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Determination of the *In Vitro* and *In Vivo* Activity of Compounds Tested Against Punta Toro Virus.

Final Report

Robert W. Sidwell, Ph.D.

January 28, 1991

Supported by

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Military Relevance: The viruses of military significance targeted by this research are sandfly fever virus and Rift Valley fever virus, both endemic to the Middle Eastern area and capable of severely hampering military operations if an outbreak occurs in susceptible populations. The Punta Toro virus is a closely related virus which is safer to use in the laboratory and which, as target for antiviral agents, has been shown to be highly predictable of efficacy against sandfly and Rift Valley fever viruses. ***In Vivo* Assessment of Lethal Toxicity:** Approximate LD50 values were obtained in mice for 105 AVS compounds. ***In Vitro* Evaluation of Test Compounds Against Punta Toro Virus:** A total of 342 AVS compounds were evaluated against the Adames strain of PTV using an *in vitro* assay procedure. This procedure used, as initial endpoint, inhibition of viral-induced cytopathic effect in LLC-MK_2 cells, and as a confirming test, reduction in virus yield. 15 compounds were considered to be strongly active against the virus; most were also inhibitory to the Balliet virus strain in confirming experiments. A total of 42 compounds exerted a moderate PTV-inhibitory effect. **Effect of AVS Compounds on Hepatotropic Infections in Mice Induced by the Adames Strain of Punta Toro Virus:** A total of 882 experiments were run in evaluating 121 AVS compounds against the hepatotropic PTV infection. Ribavirin (AVS01) and six chemical derivatives were considered markedly effective and acting specifically against the virus infection. A total of 23 immunomodulating substances also had strong anti-PTV effects. An apparent common immunological property among

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19. ABSTRACT

the latter PTV inhibitors was the induction of IFN by each compound. **Effect of AVS Compounds on Neurotropic Infections Induced by the Balliet Strain of Punta Toro Virus:** A total of 51 AVS substances were evaluated in one or more experiments against the fatal neurologic infection induced by the Balliet strain of PTV in mice. Thirteen compounds (AVS01, 02, 206, 253, 1754, 2149, 2776, 3588, 3589, 3934, 5896, 6080, 6082) exerted some degree of inhibition of this infection, but none were considered markedly active. **Effects of Drug Combinations on the Hepatropic Punta Toro Virus Infection in Mice:** A total of 9 drug combinations were evaluated against the hepatotropic PTV infection in vivo. The combinations of AVS01 + AVS2149, AVS01 + AVS2776, AVS01 + AVS5587, and AVS206 + AVS1767 were considered synergistic in their action. AVS01 + AVS1754 had additive or slightly synergistic effects. The combinations of AVS01 + AVS2779 and AVS01 + AVS1761 were considered antagonistic in their action. AVS206 + AVS2776 appeared antagonistic, but due to some questionable data will be repeated. **Comparisons of the Anti-Punta Toro Virus Efficacy of AVS01, AVS02, and AVS206:** Extensive in vivo studies were run in parallel to compare the anti-PTV efficacy of AVS01, 02, and 206. Questions considered were: Which had the greater therapeutic index when given s.c. or p.o.; which was most effective when s.c. or p.o. treatment was delayed; which was most effective when s.c. or p.o. treatment duration was reduced; which was most effective p.o. when viral challenge was increased; which was most effective against the i.c. inoculated Balliet strain of PTV; which, when administered p.o., would better control daily development of hepatic icterus, virus titers in various tissues, and the usual PTV-associated decline in white blood cells? Although the results were not fully definitive, we conclude that AVS206 was marginally better than AVS01 or AVS02. **Characterization of the Adames Strain Punta Toro Virus Infection in Mice:** The disease induced by s.c. or i.p. inoculation of the Adames strain of PTV into 3 week-old C57BL/6 mice is acute, characterized by rapid rise of virus in all tissues and in blood, major liver failure within 3-5 days of infection, and subsequent death of the animal by 3 to 6 days. The infection is profoundly immunosuppressive, as seen by decreasing total splenic T and B cells, reduced B and T cell function, and declining ability of splenic cells to produce IL-2. The cell decreases were seen by day 3 of the infection, the functionality declines by day 1. Older mice, Swiss Webster and NIH-III mice do not appear to be as sensitive to the disease as the C57BL/6 strain. The infection is apparently not readily transmitted between cages. **Effects of Punta Toro Virus on Macromolecular Synthesis of Cells:** Punta Toro virus infection appeared to significantly inhibit DNA, RNA and protein synthesis from 16-24 hours post-virus exposure. DNA synthesis, as reflected by deoxyadenosine uptake, remains perturbed throughout PTV infection from 8-48 hours post virus exposure. In addition, PTV seems to enhance macromolecular synthesis 1 hour post exposure to virus in log phase cells. Whether these effects are an actual stimulation or depression of macromolecular synthesis due to viral-induced stimulation, or inhibition of cellular enzymes, to viral-induced enzymes, or to an increase or decrease in cell permeability is still to be determined. **Investigations into the In Vitro Efficacy of AVS206 Against Punta Toro Virus:** AVS01 and AVS206, when used in combination vs Adames PTV in an *in vitro* experiment, appeared to have an indifferent or partial antagonistic effect. These data suggest the compounds are probably acting by similar mechanisms and may be competing with each other. The *in vitro* anti-PTV activity of AVS206 was reversed by adenosine, 2-deoxyadenosine, guanosine, guanosine 5'-PO_4, and 2-deoxyguanosine. Other precursors, including inosine, adenine, cytidine, thymidine, uridine, and xanthosine did not have a noticeable reversal effect. The anti-PTV activity of AVS01 was reversed by guanosine but not by xanthosine. Neither AVS206 nor AVS01 were considered strongly cytotoxic as measured by effects on DNA, RNA, and protein synthesis. AVS206 was less inhibitory to DNA synthesis as measured by uptake of ^{32}P than AVS01. Our data suggest both materials to have a static, rather than toxic, effect on LLC-MK_2 cells. AVS206 and AVS01 were subjected to enzymatic degradation with adenosine deaminase. AVS206 was broken down to a species that comigrated with AVS01 as determined by silica gel thin layer chromatography. AVS01 was unaffected by deaminase treatment. In addition, with prolonged incubation, AVS206 apparently degenerates to a species that also comigrates with AVS01. **Effects of Treatment with AVS206 on Delayed Infection Parameters in Punta Toro Virus-Infected Mice:** AVS206 administered p.o. twice daily for 3 days was highly effective vs Adames PTV infections in mice, and, once treatment was terminated, detectable infectious virus did not return to either livers or sera from surviving mice up to 4 days later. **Comparisons of the Biochemical Cytotoxicity of AVS01, 111, 253, 257, and 3706:** AVS111, 253, 257, and 3706 were considered more cytotoxic to LLC-MK2 cells than AVS01 as measured by effects on DNA, RNA, and protein synthesis. **A Measurement of AVS01 Toxicity Using Pulse Oximetry:** Ribavirin administered i.p. twice a day for 5 days in doses of 800 and 1200 mg/kg/day was lethally toxic to 4 week-old BALB/c mice. As the animals approached the time of death, which was attributed to excessive hemorrhaging in the gut, their arterial oxygen saturation (SaO_2%) declined appreciably. **Overview of *In Vivo* Anti-Punta Toro Virus Activity of AVS Compounds: Summary of Five Years' Testing. Presentations and Publications:** A total of 15 presentations were made to various scientific meetings during this contract. Fourteen papers have been published or submitted to scientific journals.

SUMMARY

1. The viruses of military significance targeted by this research are sandfly fever virus and Rift Valley fever virus, both endemic to the Middle Eastern area and capable of severely hampering military operations if an outbreak occurs in susceptible populations. The Punta Toro virus is a closely related virus which is safer to use in the laboratory and which, as target for antiviral agents, has been shown to be highly predictable of efficacy against sandfly and Rift Valley fever viruses.

2. Approximate LD50 values were obtained in mice for 105 AVS compounds.

3. A total of 342 AVS compounds were evaluated against the Adames strain of PTV using an *in vitro* assay procedure. This procedure used, as initial endpoint, inhibition of viral-induced cytopathic effect in LLC-MK_2 cells, and as a confirming test, reduction in virus yield. 15 compounds were considered to be strongly active against the virus; most were also inhibitory to the Balliet virus strain in confirming experiments. A total of 42 compounds exerted a moderate PTV-inhibitory effect.

4. A total of 882 experiments were run in evaluating 121 AVS compounds against the hepatotropic PTV infection. Ribavirin (AVS01) and six chemical derivatives were considered markedly effective and acting specifically against the virus infection. A total of 23 immunomodulating substances also had strong anti-PTV effects. An apparent common immunological property among the latter PTV inhibitors was the induction of IFN by each compound.

5. A total of 51 AVS substances were evaluated in one or more experiments against the fatal neurologic infection induced by the Balliet strain of PTV in mice. Thirteen compounds (AVS01, 02, 206, 253, 1754, 2149, 2776, 3588, 3589, 3934, 5896, 6080, 6082) exerted some degree of inhibition of this infection, but none were considered markedly active.

6. A total of 9 drug combinations were evaluated against the hepatotropic PTV infection in vivo. The combinations of AVS01 + AVS2149, AVS01 + AVS2776, AVS01 + AVS5587, and AVS206 + AVS1767 were considered synergistic in their action. AVS01 + AVS1754 had additive or slightly synergistic effects. The combinations of AVS01 + AVS2779 and AVS01 + AVS1761 were considered antagonistic in their action. AVS206 + AVS2776 appeared antagonistic, but due to some questionable data will be repeated.

7. Extensive in vivo studies were run in parallel to compare the anti-PTV efficacy of AVS01, 02, and 206. Questions considered were: Which had the greater therapeutic index when given s.c. or p.o.; which was most effective when s.c. or p.o. treatment was delayed; which was most effective when s.c. or p.o. treatment duration was reduced; which was most effective p.o. when viral challenge was increased; which was most effective against the i.c. inoculated Balliet strain of PTV; which, when administered p.o., would better control daily development of hepatic icterus, virus titers in various tissues, and the usual PTV-associated decline in white blood cells? Although the results were not fully definitive, we conclude that AVS206 was marginally better than AVS01 or AVS02.

8. The disease induced by s.c. or i.p. inoculation of the Adames strain of PTV into 3 week-old C57BL/6 mice is acute, characterized by rapid rise of virus in all tissues and in blood, major liver failure within 3-5 days of infection, and subsequent death of the animal by 3 to 6 days. The infection is profoundly immunosuppressive, as seen by decreasing total splenic T and B cells, reduced B and T cell function, and declining ability of splenic cells to produce IL-2. The cell decreases were seen by day 3 of the infection, the functionality declines by day 1. Older mice, Swiss Webster and NIH-III mice do not appear to be as sensitive to the disease as the C57BL/6 strain. The infection is apparently not readily transmitted between cages.

9. Punta Toro virus infection appeared to significantly inhibit DNA, RNA and protein synthesis from 16-24 hours post-virus exposure. DNA synthesis, as reflected by deoxyadenosine uptake, remains perturbed throughout PTV infection from 8-48 hours post virus exposure. In addition, PTV seems to enhance macromolecular synthesis 1 hour post exposure to virus in log phase cells. Whether these effects are an actual stimulation or depression of macromolecular synthesis due to viral-induced stimulation, or inhibition of cellular enzymes, to viral-induced enzymes, or to an increase or decrease in cell permeability is still to be determined.

10. AVS01 and AVS206, when used in combination vs Adames PTV in an *in vitro* experiment, appeared to have an indifferent or partial antagonistic effect. These data suggest the compounds are probably acting by similar mechanisms and may be competing with each other. The *in vitro* anti-PTV activity of AVS206 was reversed by adenosine, 2-deoxyadenosine, guanosine, guanosine 5'-PO_4, and 2-deoxyguanosine. Other precursors, including inosine, adenine, cytidine, thymidine, uridine, and xanthosine did not have a noticeable reversal effect. The anti-PTV activity of AVS01 was reversed by guanosine but not by xanthosine. Neither AVS206 nor AVS01 were considered strongly cytotoxic as measured by effects on DNA, RNA, and protein synthesis. AVS206 was less inhibitory to DNA synthesis as measured by uptake of ^{32}P than AVS01. Our data suggest both materials to have a static, rather than toxic, effect on LLC-MK_2 cells. AVS206 and AVS01 were subjected to enzymatic degradation with adenosine deaminase. AVS206 was broken down to a species that comigrated with AVS01 as determined by silica gel thin layer chromatography. AVS01 was unaffected by deaminase treatment. In addition, with prolonged incubation, AVS206 apparently degenerates to a species that also comigrates with AVS01.

11. AVS206 administered p.o. twice daily for 3 days was highly effective vs Adames PTV infections in mice, and, once treatment was terminated, detectable infectious virus did not return to either livers or sera from surviving mice up to 4 days later.

12. AVS111, 253, 257, and 3706 were considered more cytotoxic to LLC-MK2 cells than AVS01 as measured by effects on DNA, RNA, and protein synthesis.

13. Ribavirin administered i.p. twice a day for 5 days in doses of 800 and 1200 mg/kg/day was lethally toxic to 4 week-old BALB/c mice. As the animals approached the time of death, which was attributed to excessive hemorrhaging in the gut, their arterial oxygen saturation (SaO_2%) declined appreciably.

14. Overview of *In Vivo* Anti-Punta Toro Virus Activity of AVS Compounds: Summary of Five Years' Testing.

15. Presentations and publications: A total of 15 presentations were made to various scientific meetings during this contract. Fourteen papers have been published or submitted to scientific journals.

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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TABLE OF CONTENTS

	Contents	Page
Summary		1
Foreword		3
I.	Military Relevance	5
II.	*In Vivo* Assessment of Lethal Toxicity	7
III.	*In Vitro* Evaluation of Test Compounds Against Punta Toro Virus	14
IV.	Effect of AVS Compounds on Hepatotropic Infections in Mice Induced by the Adames Strain of Punta Toro Virus	21
V.	Effect of AVS Compounds on Neurotropic Infections Induced by the Balliet Strain of Punta Toro Virus	29
VI.	Effects of Drug Combinations on the Hepatropic Punta Toro Virus Infection in Mice	33
VII.	Comparisons of the Anti-Punta Toro Virus Efficacy of AVS01, AVS02, and AVS206	43
VIII.	Characterization of the Adames Strain Punta Toro Virus Infection in Mice	77
IX.	Effects of Punta Toro Virus on Macromolecular Synthesis of Cells	96
X.	Investigations into the In Vitro Efficacy of AVS206 Against Punta Toro Virus	100
XI.	Effects of Treatment with AVS206 on Delayed Infection Parameters in Punta Toro Virus-Infected Mice	116
XII.	Comparisons of the Biochemical Cytotoxicity of AVS01, 111, 253, 257, and 3706	118
XIII.	A Measurement of AVS01 Toxicity Using Pulse Oximetry	127
XIV.	Overview of *In Vivo* Anti-Punta Toro Virus Activity of AVS Compounds: Summary of Five Years' Testing	130
XV.	Presentations and Publications	150

I. MILITARY RELEVANCE

The Punta Toro virus (PTV) is a *Phlebovirus* in the Bunyaviridae family of viruses, and is closely related to sandfly fever (SF) virus and Rift Valley fever (RVF) virus. Both SF and RVF viruses are considered important viruses militarily.

Sandfly fever: During World War II, approximately 19,000 members of the Allied armed forces in the Middle Eastern area were afflicted with SF infections, with most requiring hospitalization (1, 2). From 3% to 10% of all troops were afflicted with the disease at that time, with some units reporting attack rates of over 50% (3). These rates were especially high in the Persian Gulf command, reaching a peak of 235 cases/1000 men (1).

Oldfield et al. (3), in a recent review indicating the potential importance of SF in the current Iraqi conflict, stated the following concerning the further military significance of SF:

> "The military significance of sandfly fever is magnified because of its short incubation period, which can render large numbers of nonimmune troops ineffective early in an operation, while the endemic forces would be largely immune and unaffected."

The disease has a sudden onset and intense symptoms of fever, severe frontal headache with retro-orbital pain associated with severe myalgias, and often nausea, vomiting, abdominal pain and diarrhea (4, 5). These disease manifestations persist 2-4 days. The disease is transmitted by *Phlebotumus papatasi*, a nocturnal biting midge which is especially abundant in the Middle East from June to August (6).

Rift Valley fever: Severe epidemics of RVF have been reported since 1930 throughout much of the African area. An outbreak occurred in Sudan in 1976 (7), presumably with the disease spreading to Egypt in 1977-78 which resulted in an estimated 200,000 human cases and at least 600 deaths (8, 9). In the epidemic areas, the human infection rates were as high as 35% (8). In the last 10 years, there have been several outbreaks in the sub-Saharan Africa, the most recent being an ongoing epidemic in Mauritania (10).

The RVF disease often resembles human influenza, with abrupt onset of fever and associated symptoms lasting 2-5 days. Some cases may be more serious or fatal, resulting from liver necrosis with hemorrhagic phenomena, retinitis with visual impairment, and meningoencephalitis (11, 12).

The RVF virus can be transmitted by a variety of mosquito species (13), and infects many domestic animals. Because of this insect transmission and the movement of vertebrates potentially carrying the virus, it has the potential to be spread to distant geographic sites. In view of the close proximity of Egypt and Sudan to Saudi Arabia, the potential for Allied forces contracting this significant virus disease appears very real.

Punta Toro virus: This virus, as pointed out at the beginning of this section, is closely related to both SF and RVF viruses, and like those viruses, is also transmitted by biting insects. The virus is of particular value because is induces a disease very similar to that induced by RVF in mice, but causes a less severe disease in man and is not readily transmitted in the laboratory. PTV, RVF, and SF viruses all appear quite similarly sensitive to the same antiviral compounds (14-20, unpublished findings reported by Drs. J. Huggins and M. Kende of the U.S. Army Medical Research Institute for Infectious Diseases).

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II. *IN VIVO* ASSESSMENT OF LETHAL TOXICITY

Introduction

Before compounds submitted to us can be evaluated for *in vivo* PTV activity, information is needed regarding the approximate LD50 of those compounds as determined using the same treatment schedule to be used in the antiviral experiments. This report describes the results of all toxicity experiments in which death was used as an endpoint. In some cases, due to lack of sufficient compound, an *in vivo* antiviral experiment was run without preliminary toxicity data. In all *in vivo* antiviral experiments, toxicity controls were run in parallel, so those data were also included in this section. The results will hopefully provide sufficient information on the murine toxicity of the AVS compounds that other investigators will be able to run antiviral studies with appropriate dosages of the compounds.

Materials and Methods

Compounds: All compounds were submitted to us by Technassociates and later by Biological Research Faculty & Facility, Inc. (Rockville, MD). The compounds were weighed and dissolved or suspended in vehicles considered most appropriate for the compound. These vehicles were physiological saline, sterile water for injection, 4% carboxy methylcellulose, or dimethylsulfoxide, the latter used for compounds given intravenously.

Animals: C57BL/6 mice 3-4 weeks of age were obtained from Simonsen Laboratories (Gilroy, CA). All were quarantined at least 24 hr prior to use, and maintained on Wayne Lab Blox mouse chow and tap water *ad libitum.* They were caged in shoe box style polycarbonate cages with Sani-cell bedding used. All were housed 5 to a cage.

Toxicity Assessments: Mice were injected with varying 2-fold dilutions according to the indicated treatment regimens. All were weighed immediately prior to treatment and again 18 hr after the final treatment to determine if normal weight gain occurred. In preliminary toxicity studies, the mice were held a total of 14 days. When used as parallel toxicity controls in PTV studies, the animals were held a total of 21 days. Five mice were used at each dosage level. The volume administered was 0.01 ml/g of body weight. Parameters for evaluation included weight change, obvious signs of distress such as diarrhea, prostration, or tremors, and death, which was noted daily. The LD50 dose was calculated by the Reed-Muench method (1).

Results and Discussion

The toxicity determinations, expressed as LD50 values, are summarized in Table I-1. A total of 105 compounds were evaluated over the 5-year period of this contract. In some cases ">" values are shown because we had not achieved a lethal dose and no further studies were run due to inadequate compound. Values shown as "~" were estimated based on the observation that slightly lower doses were lethal, but to less than 50% of the animals, or treatment with lower doses caused marked weight loss in the animals, suggesting the maximum tolerated dose (MTD) had essentially been reached.

Conclusions

Approximate LD50 values were obtained in mice for 105 AVS compounds.

Literature Cited

1. Reed, L.J. and H. Muench. (1938) A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27:493-497.

Table II-1. Preliminary Toxicity Evaluations of AVS Compounds[a]

Compound (AVS No.)	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
01	bid x 5	s.c.	560
	bid x 5	p.o.	1300
	once only	s.c.	>1000
02	bid x 5	s.c.	1700
	bid x 5	p.o.	2252
52	once only	s.c.	~2000
	tid x 5	p.o.	~800
	tid x 5	s.c.	~800
65	once only	s.c.	~1200
	bid x 5	s.c.	1500
	tid x 5	s.c.	~700
	tixd x 5	i.p.	~700
79	tid x 5	i.p.	225
	once only	p.o.	~900
	qd x 5	s.c.	300
	bid x 5	i.p.	150
	bid x 5	p.o.	>200
111	bid x 5	s.c.	~3000
	bid x 5	p.o.	7750
	once only	s.c.	~3000
147	bid x 5	s.c.	~3000
	once only	p.o.	~1500
	tid x 5	s.c.	>500
167	bid x 5	s.c.	~2000
206	bid x 5	s.c.	1700
	bid x 5	p.o.	2600
212	tid x 5	s.c.	~300
215	bid x 5	i.p.	~800
	qd x 5	s.c.	~150
222	bid x 5	s.c.	~3000
	tid x 5	s.c.	>500
	once only	i.p.	~1800
	bid x 5	s.c.	~2200
233	bid x 5	s.c.	~900
	once only	s.c.	~3600
253	qd x 5	i.p.	~1200
	bid x 5	i.p.	~1200
	bid x 5	p.o.	>500

Compound (AVS No.)	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
272	qd x 5	s.c.	~600
	bid x 5	s.c.	375
347	bid x 5	s.c.	90
360	bid x 5	s.c.	>500
1018	once only	p.o.	>12.5
	e 4 days x 3	p.o.	>12.5
1212	bid x 5	s.c.	~600
	once only	s.c.	~600
	once only	i.p.	~800
1754	once only	i.p.	250
	once only	p.o.	~300
1761	qd x 8	i.p.	~8
1767	bid x 5	s.c.	3000
	bid x 5	i.p.	~800
1777	once only	i.p.	~15
	bid x 5	i.p.	0.75
	bid x 5	s.c.	0.3
1778	once only	s.c.	~100
	bid x 5	i.p.	200
	bid x 5	s.c.	200
1968	e 4 days x 3	p.o.	>100
	once only	p.o.	~200
1969	once only	p.o.	>200
	once only	i.p.	>200
	bid x 5	p.o.	>200
	bid x 5	i.p.	>100
2149	once only	i.p.	~20
	bid x 5	i.p.	~20
2563	bid x 5	s.c.	>75
	qd x 5	s.c.	~500
2605	bid x 5	s.c.	>220
2700	bid x 5	i.p.	300
	once only	i.p.	~600
2712	qd x 5	i.p.	>144 µg/kg/day
	once only	i.p.	>200 µg/kg/day
2713	qd x 5	i.p.	>36 µg/kg/day
2716	bid x 5	s.c.	>300
2741	bid x 5	s.c.	>2000
2742	bid x 5	s.c.	>2000

Compound (AVS No.)	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
2776	qd x 3	p.o.	~800
	once only	p.o.	>800
	qd x 3	i.p.	~500
	once only	i.p.	~500
2777	qd x 3	p.o.	>400
	qd x 3	i.p.	~500
	once only	i.p.	>400
2778	qd x 3	i.p.	~600
	qd x 3	p.o.	>400
	once only	p.o.	>400
2779	once only	i.p.	>200
	once only	p.o.	>200
	qd x 3	i.p.	~150
2786	bid x 5	s.c.	>300
2812	bid x 5	s.c.	4.7
	bid x 5	i.p.	~5
	qd x 5	s.c.	6.5
	qd x 5	i.p.	~5
2880	qd x 3	i.p.	~50
	bid x 3	i.p.	~50
	bid x 5	p.o.	~50
	qd x 2	p.o.	>50
2885	bid x 5	i.p.	~800
	bid x 5	s.c.	>400
2933	once only	i.p.	>10,000
	eod x 3	i.p.	>1000
2978	bid x 7	i.p.	>400
2980	once only	i.p.	~100
	bid x 5	i.p.	~15
3425	bid x 5	i.p.	550
	once only	s.c.	~500
	qd x 5	s.c.	~350
3580	bid x 5	i.p.	>100
3585	once only	i.p.	>400
3587	once only	p.o.	>500
	once only	i.p.	600
	qd x 3	i.p.	500
3588	once only	i.p.	~600
	qd x 3	i.p.	>400
3589	once only	p.o.	>500
	once only	i.p.	>400
	qd x 3	i.p.	>400

Compound (AVS No.)	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
3593	tid x 5	i.p.	>150
	bid x 6	i.p.	>150
	once only	i.p.	>500
	ad lib	p.o. (drink water)	>93
3679	bid x 5	s.c.	>400
3706	bid x 5	s.c.	>2000
	bid x 5	s.c.	~3000
3925	once only	i.p.	150
	qd x 5	i.p.	19
	eod x 3	i.p.	>40
3926	once only		75
	bid x 5		>40
3927	once only	i.p.	180
	bid x 5	i.p.	>40
3933	qd x 5	i.p.	>250
3934	qd x 7	i.p.	>1000
	bid x 7	p.o.	>600
	bid x 7	i.p.	>300
	qd x 7	p.o.	>300
3960	bid x 5	s.c.	>900
	bid x 7	i.p.	>600
	bid x 7	p.o.	>800
4071	bid x 5	s.c.	>500
4113	qd x 5	s.c.	>12
4206	bid x 5	s.c.	>400
	bid x 5	i.p.	>600
4272	bid x 5	i.p.	~16
	bid x 5	s.c.	~50
	bid x 5	p.o.	~150
	once only	i.p.	~15
4273	bid x 5	s.c.	~100
	once only	i.p.	~250
4282	eod x 6	i.p.	0.7
	once only	i.p.	0.3
	qd x 3	i.p.	0.5
4283	once only	i.p.	>260
4284	once only	i.p.	>180
4285	once only	i.p.	>100
4286	once only	i.p.	>200
4287	once only	i.p.	>200

Compound (AVS No.)	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
4588	bid x 5	s.c.	>400
	bid x 5	i.p.	>600
4593	once only	i.p.	>200
4616	bid x 5	s.c.	>150
4617	bid x 5	s.c.	>800
4618	bid x 5	s.c.	>400
4796	bid x 5	s.c.	>300[c]
4855	bid x 5	s.c.	>500
5058	bid x 5	s.c.	>500
5079	qd x 5	i.p.	>25,000 units
5087	qd x 5	i.p.	>150
	once only	i.p.	~400
5582	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~700[b]
5587	bid x 1	i.p.	~200
5591	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~600[b]
5786	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	50[b]
5896	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	90[b]
5897	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	280[b]
5898	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	90[b]
6080	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	89[b]
6081	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	28[b]
6082	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~104[b]
6083	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	28[b]
6290	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	158[b]
6291	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~104[b]

Compound (AVS No.)	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
6292	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	~104[b]
6296	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	104[b]
6297	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	32[b]
6299	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	158[b]
6300	qd x 5	i.v. (days 1,3,5) i.p. (days 2,4)	104[b]
6334	bid x 5	i.p.	>250
6337	bid x 5	i.p.	~350
6417	bid x 5	i.p.	188
6477	bid x 5	i.p.	>100
6501	bid x 5	i.p.	>250

[a]10-13 g C57BL/6 mice.

[b]15-20 g C57BL/6 mice.

[c]Highly insoluble compound, so data are suspect. It became a taffy-like mass in saline, which partially dissolved in 0.6 ml MeOH. Addition of DMSO did not appear to help.

III. *IN VITRO* EVALUATION OF TEST COMPOUNDS AGAINST PUNTA TORO VIRUS

Introduction

An initial phase of our anti-Punta Toro virus (PTV) testing program for the first 3 years of this contract was to evaluate new compounds *in vitro* for activity against the Adames strain of this virus. In the initial experiments, inhibition of cytopathic effect (CPE) was determined. Compounds exhibiting adequate CPE inhibition (Virus Rating [VR] ≥ 0.5) were retested and their effects on virus yield (virus titer reduction [VTR] at the maximum tolerated dose [MTD]) was also determined. Active compounds were then also evaluated against the Balliet strain of PTV.

This section summarizes the results of these 3 years' testing, with the compounds categorized according to degree of *in vitro* antiviral activity seen.

Materials and Methods

Virus: Twice plaque isolated PTV, both Adames and Balliet strains, were prepared in LLC-MK_2 cells as described by us (1).

Cells: LLC-MK_2 (Rhesus monkey kidney) cells were used. They were initially obtained from the American Type Culture Collection (ATCC, Rockville, MD). Various passages of cells were used over the 3-year period of this study. Growth medium was minimum essential medium (MEM, Gibco Labs, Grand Island, NY) containing 5% fetal bovine serum (FBS, HyClone Labs, Logan, UT) and 0.1% $NaHCO_3$ without antibiotics. All were determined to be mycoplasma-free.

Test Compounds: All materials were provided by the USAMRIID contractor (Technassociates and later by Biological Research Faculty and Facility, Inc., Rockville, MD) for these tests. Each was stored and handled according to instructions from that supplier.

In Vitro Testing Procedures: Seven concentrations of test compound, these concentrations usually being 1000, 320, 100, 32, 10, 3.2 and 1 µg/ml, were added in 0.1 ml amounts to an 18-hr monolayer of cells in 96-well flat-bottom microplates. Adames strain PTV (320 CCID50/ml) was added in 0.1 ml volume 15 minutes later. Three virus-containing cups in each microplate were used for each compound dosage level, with one cup used for toxicity controls (cells + sterile virus diluent + compound). Six cups in each panel were used for virus controls (cells + virus + drug diluent) and 6 cups in each panel were used for normal cell controls (cells + sterile virus diluent + drug diluent). Test medium in which virus and compound were suspended or dissolved was MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg/ml gentamicin. Viral CPE was graded from 0 (normal cells) to 4 (virtually complete destruction of the cell layer) 6-7 days post-virus exposure. The CPE was read by an individual who was well trained for CPE evaluation, then this reading was confirmed by a second, similarly trained individual.

Reduction in CPE was evaluated by VR as we have described previously (1, 2) and by 50% effective dose (ED50). The VR is a numerical expression of antiviral activity, taking into account percent of CPE inhibition and partial cytotoxicity of the test compound. In our experience a VR of 1.0 is indicative of definite antiviral activity, a VR of 0.5 - 0.9 indicates moderate activity, and a VR of <0.5 suggests slight activity perhaps resulting from cytotoxicity only. The ED50 was determined by plotting percent CPE inhibition vs test compound concentration, with the ED50 level being that level causing an approximate 50% CPE inhibition. Also included in the test was an estimated maximum tolerated dose (MTD) of the test compound, this MTD being the lowest dosage causing visually discernible cytotoxic effects in the concurrently run toxicity controls. Cytotoxicity was determined by microscopic assessment of compound-induced cytopathic effects in treated cultures compared with those in control cells run in the same plate.

Validation of apparent positive activity was done in early *in vitro* experiments by fixing the drained cells in 10% formalin and staining them with 1% crystal violet, which clearly demonstrated the complete cell monolayer. The stained plate was labeled and photographed. The experiment was then repeated and, in addition to CPE inhibition being determined, virus yield was also determined by freezing the plate, thawing at room temperature, and the medium from each 10-fold dilution and from virus controls removed and virus quantified. The virus quantification was done by end-point dilution, determining CPE induced in triplicate cups containing LLC-MK_2 cells exposed to 0.1 ml of 10-fold dilutions of each sample collected.

As positive control, ribavirin (AVS01) was tested in parallel in each series of tests. This compound was shown by us (Section I, First Annual Report) and (1) to be highly active vs PTV *in vitro* and *in vivo*.

Compounds exhibiting confirmed positive activity in these tests were retested in a similar manner using the Balliet strain of virus. These latter experiments are also described in this Section.

Results and Discussion

A total of 342 compounds were evaluated. Those considered highly active vs PTV are summarized in Table III-1.

Table III-2 summarizes the activity of those AVS compounds considered moderately effective against PTV. Again, activity against both Adames and Balliet virus strains are shown, when tested. Since we were instructed to terminate in vitro antiviral testing with PTV midway through the 3rd year of this contract, some followup testing was not completed.

Compounds considered inactive vs PTV, i.e., having VR's of less than 0.5, are listed in Table III-3.

Often seen in the tables is a rather wide range of antiviral activity and cytotoxicity with a particular compound. This was often due to special procedures taken in repeated tests to make a somewhat insoluble compound more soluble in the test medium. This sometimes involved warming the solution, adding acid or base, sonifying, or adding small quantities of dimethyl sulfoxide. When more of the compound was in solution, this often both lowered the ED50 and the MTD, but this lowering did not always occur at the same rate.

Few compounds had a major effect on lowering virus yield in the VTR portion of confirming experiments. Such an assay is rather a severe test for a compound, especially when the compound is added to the cells 15 minutes after the virus, so that a complete arrest of virus replication is difficult to achieve. However, when compounds do reduce the virus titers by one or more $\log_{10}$, it is considered to be a strongly active lead.

It is important to note that the activity against the Balliet strain was not always the same as against the Adames strain. It is well recognized that various virus strains of many viruses will differ significantly in their sensitivity to antiviral agents. Since a goal in this program is to discover and develop compounds active against both Adames and Balliet viruses, compounds shown in these *in vitro* experiments to be significantly inhibitory to both viruses should be particularly considered for *in vivo* testing against infections induced by both viruses.

Conclusions

A total of 342 AVS compounds were evaluated against the Adames strain of PTV using an *in vitro* assay procedure. This procedure used, as initial endpoint, inhibition of viral-induced cytopathic effect in LLC-MK_2 cells, and as a confirming test, reduction in virus yield. Fifteen compounds were considered to be strongly active against the virus; most were also inhibitory to the Balliet virus strain in confirming experiments. A total of 42 compounds exerted a moderate PTV-inhibitory effect.

Literature Cited

1. Sidwell, R. W., J. H. Huffman, B. B. Barnett, and D. Y. Pifat. 1988. *In vitro* and *in vivo phlebovirus* inhibition by ribavirin. Antimicrob. Ag. Chemother. 32:331-336.
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Table III-1. AVS Compounds Considered Highly Inhibitory (VR of 1.0 or >) to Punta Toro Virus In Vitro.

	Adames Strain					Balliet Strain	
Compound (AVS No.)	VR[a] (range)	ED50[b] (µg/ml) (range)	MTD[c] (µg/ml) (range)	VTR[d] at MTD (log_{10})	Max TI	VR Range	Max TI
01	0.9-1.2	4-10	3.2-100	2.7	3.3	0.9-1.2	5.7
052	1.4-1.6	1	3.2	0.7	3.2	1-1.2	10.7
148	1.4-2.0	0.018	0.001	0.0	0.6	0.6-0.7	3.2
206	1.0-1.2	8-16	32	4.6	4	0.8-1.0	3.7
215	1.3	5	10	2.5	2	0.6-0.8	3.2
253	0.9-1.2	0.1-10	0.01	1.0	0.1	0.6-0.8	3.2
1089	0.8-1.4	0.16	0.32-3.2	—	3.2	1.2-1.4	0.7
1754	0.7-1.7	<1.0-72	32-100	0.5	>100	0.3-0.6	7.7
2700	0.7-1.0	10-56	1.0-3.2	0.0	0.1	0.2	0.01
3038	≥1.0-1.2	0.096-1.0	0.01-3.2	0.2	3.2	1.3-1.8	0.1
3593	0.6-1.2	0.9	0.32	0.5	0.4	—	—
3705	0.9-1.1	2.2-3.5	1	0.5	0.5	0.6-1.1	0.4
4785	0.9-1.1	4.6-9.5	1-32	—	7.0	1.0	5.4
4796	1.5	2.4	32	—	13.3	—	—
4798	0.6-1.4	0.1	0.32	—	3.2	—	—

[a]Virus rating

[b]50% Effective (virus-inhibitory) dose.

[c]Minimum toxic dose.

[d]Virus titer reduction determined at MTD.

[e]Therapeutic index (MTD÷ED50).

Table III-2. AVS Compounds Considered Moderately Inhibitory (VR of 0.5-0.9) to Punta Toro Virus In Vitro.

	Adames Strain					Balliet Strain	
Compound (AVS No.)	VR[a] (range)	ED50[b] (µg/ml) (range)	MTD[c] (µg/ml) (range)	VTR[d] at MTD (log_{10})	Max TI	VR Range	Max TI
78	0-1.0	>10-21	<3.2-10	—	0.5	—	—
111	0.4-0.8	18-130	3.2	0.0	0.18	0.4	0.02
136	0.4-0.5	85-270	100	0.5	1.2	0.2	0.07
139	0.2-0.8	16-24	3.2	—	0.2	0.8	0.04
195	0.7-0.9	50-55	32	2.2	0.6	—	—
200	0.3-0.6	50	10	0.0	0.2	—	—
212	0.6	45-82	32-100	0.0	1.2	0.2	0.2
233	0.7-0.8	25-28	3.2-10	0.0	0.4	0.2	0.03
257	0.7-1.0	4-31	3.2	—	0.8	0.5-0.6	0.05
347	0.5-0.8	6	1.0	0.2	0.2	0.6	0.2
361	0.6-0.8	2	1.0	0.5	0.5	0.6	0.5
1159	0.6	22-54	10-32	0.3	0.6	0.3-0.5	1.1
1160	0.6-0.7	24-55	3.2-10	—	0.6	0.3	0.1
1850	0.3-0.7	22-70	10-32	—	0.5	—	—
1976	0.6-0.7	25-45	3.2-10	—	0.2	0.4	0.4
1978	0.4-0.5	520-1000	320-1000	2.0	1.0	—	—
2296	0.6	240	32	0.2	0.1	0.1	0.01
2301	0.8	9.5	3.2	0.0	0.3	0.4	0.05
2543	0.4-0.7	20-30	3.2-10	0.2	0.5	—	—
2563	0.6-0.8	5-7.5	1.0-10	—	2	0.4	0.6
2714	0.3-0.6	9-10	1-3.2	0.0	0.4	—	—
2716	0.2-0.9	9-46	1-10	0.0	0.3	—	—
2811	0.7	1-3	1-3.2	0.1	3.2	0.8	0.5

Compound (AVS No.)	Adames Strain VR[a] (range)	ED50[b] (µg/ml) (range)	MTD[c] (µg/ml) (range)	VTR[d] at MTD (log_{10})	Max TI	Balliet Strain VR Range	Max TI
2812	0.4-0.6	0.25-1.0	0.1-1	—	1.0	0.4	0.2
2869	0.5	150	320	0.3	2.1	—	—
2980	0.8	8.6	3.2	0.1	1.1	0.4-0.6	1.5
3982	0.5-0.6	60-97	320	—	5.3	0.0	0.0
4071	0.6-0.9	34-221	32-320	2.8	9.4	0.5-1.2	15.2
4074	0.6-0.9	1-1.2	0.01-3.2	—	2.7	0.5-0.8	0.4
4111	0.4-0.6	133-377	320-1000	0.3	5.1	0.3	1.7
4113	0.8-0.9	0.8-1.0	0.01-3.2	—	4.0	0.8	0.7
4116	0.3-0.5	170-649	320-1000	—	1.9	0.7	8.7
4200	0.5	1000	320	—	0.3	—	—
4235	0.6-0.7	33-54	1-10	—	0.2	0.3	0.6
4271	0.6-0.7	11-26	3.2	—	3.0	—	—
4279	0.6	5.5-5.8	≤1.0	0.0	0.2	0.0	0.0
4280	0.5-0.6	5.2-9.1	1.0	0.0	0.2	0.0	0.0
4281	0.6-0.8	2.1-2.5	1.0	1.0	0.5	0.2	0.2
4592	0.6-0.7	13-75	10-100	0.2	1.2	1.8	32
4611	0.5-0.6	77-254	100	—	1.3	1.4	36
4617	0.4-0.6	153-272	320-1000	0.5	3.7	0.6-0.7	7.7
4795	0.6	44	100	—	2.3	—	—

[a]Virus rating

[b]50% Effective (virus-inhibitory) dose.

[c]Minimum toxic dose.

[d]Virus titer reduction determined at MTD.

[e]Therapeutic index (MTD÷ED50).

Table III-3. AVS Compounds Considered Not Significantly Inhibitory[a] to Punta Toro Virus In Vitro.

AVS No.	AVS No.	AVS No.	AVSNo.	AVS No.	AVS No.
02	1970	2223	2405	2541	2745
33	1977	2224	2412	2542	2770
65	1983	2226	2413	2563	2771
68	1984	2228	2415	2565	2772
71	1985	2230	2417	2566	2773
87	1986	2234	2419	2567	2774
94	1987	2235	2421	2568	2775
95	1988	2277	2423	2569	2776
105	1989	2279	2425	2685	2777
113	1990	2282	2431	2687	2778
132	1991	2286	2442	2696	2780
167	1992	2289	2443	2697	2786
217	1995	2336	2444	2698	2787
229	1996	2340	2445	2699	2788
244	1998	2344	2447	2701	2789
272	2006	2361	2449	2702	2790
345	2023	2362	2450	2703	2812
653	2137	2364	2451	2704	2870
701	2138	2365	2452	2705	2871
1174	2139	2366	2453	2707	2872
1183	2140	2367	2454	2709	2873
1199	2149	2368	2456	2710	2874
1645	2159	2369	2457	2711	2875
1757	2160	2370	2458	2717	2876
1767	2188	2371	2464	2718	2877
1777	2191	2372	2477	2723	2878
1778	2193	2373	2479	2739	2879
1846	2217	2374	2484	2740	2883
1850	2219	2375	2507	2741	2884
1915	2220	2376	2538	2742	2885
1968	2221	2377	2540	2744	2886

2887	2906	2929	2959	2978	2996
2888	2907	2930	2960	2979	3035
2889	2908	2931	2961	2981	3036
2890	2909	2932	2962	2982	3037
2891	2910	2934	2963	2983	3039
2893	2911	2936	2964	2984	3585
2894	2912	2939	2965	2985	3588
2895	2913	2940	2966	2986	3589
2896	2914	2944	2967	2987	3982
2897	2915	2946	2968	2988	4111
2899	2916	2947	2969	2989	4235
2900	2917	2948	2970	2990	4279
2901	2918	2949	2971	2991	4280
2902	2919	2951	2972	2992	4281
2903	2926	2956	2973	2993	
2904	2927	2957	2974	2994	
2905	2928	2958	2975	2995	

[a]VR<0.5

IV. EFFECT OF AVS COMPOUNDS ON HEPATOTROPIC INFECTIONS IN MICE INDUCED BY THE ADAMES STRAIN OF PUNTA TORO VIRUS

Introduction

The primary thrust of this research contract is to discover and develop drugs for the treatment of experimentally induced Punta Toro virus (PTV) infections. The PTV is a *Phlebovirus* in the Bunyaviridae family which is closely related to sandfly fever (SF) and Rift Valley fever (RVF) viruses, inducers of diseases which had had a major impact in Europe, the Middle East, and Africa (1, 2), and are yet uncontrolled by antiviral drugs. PTV induces in inbred strains of parenterally inoculated mice a hepatocellular necrotic disease, leukopenia and lymphopenia which resembles the disease in man induced by SF and RVF viruses (3, and see our report in Section VIII of this report).

This section summarizes our results over the 5-year span of this contract in evaluating test substances against the PTV infection in mice. Unless otherwise directed by our Contract Officer's Technical Representative (COTR), we generally follow a relatively standard protocol in which new substances are initially tested for general toxicity in range-finding studies in mice (Section II of this report). They are then used at the maximum tolerated dose (MTD) and 2 to 3 2-fold dilutions below the MTD against a lethal infection induced by the virus. Unless otherwise instructed, our treatment regimen is subcutaneous (s.c.) treatment twice daily (bid) for 5 days beginning 4 hr pre-virus inoculation. Active compounds are then retested using expanded parameters which include death, mean survival time, liver discoloration score, serum glutamic oxalic acid and pyruvic acid transaminases (SGOT, SGPT) as indicators of liver damage, and virus titer determinations in liver homogenates and in serum. Further testing will involve determining if the compound is active orally against the infection and how long after initiation of infection can treatment be started and still render a therapeutic effect. Further followup studies may include experiments in which the efficacy of the drug is tested against increasing viral challenge. Included in all studies was the testing of a single dose of a positive control, which was ribavirin (AVS01), which has been shown to have strong anti-PTV effects (4-6).

In the preparation of this summary report, the overall activity of each active compound has been considered and the compound then categorized regarding its concluded efficacy. In many cases, insufficient compound was available for adequate follow-up studies and sometimes, due to the relative shortages of compounds, preliminary range-finding toxicity was not determined. Hence, some compounds may be categorized as having slight or no anti-PTV activity when the MTD's of the compounds had not yet been achieved. Often, certain compounds are highly dependent on the treatment protocol used; we attempt to illustrate the best means to achieve strong efficacy, but again, insufficient compound may prevent such follow-up experiments to be run.

Materials and Methods

Virus: The Adames strain of PTV was provided by DR. Dominique Pifat of USAMRIID. It was identified by Dr. Pifat as virus pool #215588, and had been safety tested by Dr. Pifat prior to being sent to us. The PTV was first isolated from the serum of A. Adames, an entomologist in the Darien Province of Panama in 1972. It was passaged twice in Vero cells prior to being sent to us. When received by us, virus was passaged 2 times through LLC-MK_2 cells, plaques isolated each time from these cells, and a large pool made from the second plaque isolate in these cells following confirmation of virus identify by serum neutralization. Later experiments, starting in the 3rd year of this project, used a new, more lethally potent PTV obtained by using low multiplicity of infection coupled with late harvest of supernate as described in Section I of our 1987 Annual Report.

Animals: Three week-old C57BL/6 mice were obtained from Simonsen Laboratories (Gilroy, CA). All weighed 10-2 g when used; heavier or lighter mice were rejected since our previous studies as well as those of Pifat and Smith (3) showed a strong difference in susceptibility with age of mouse. All were quarantined 24 to 48 hr prior to use, and maintained on Wayne Lab Blox mouse chow and tap water *ad libitum*. Female mice were used for all antiviral experiments and caged 10 to a cage; males were used for toxicity controls and held 5 to a cage.

Compounds: All compounds were submitted to us by Technassociates and later by Biological Research Faculty & Facility, Inc. Compounds were usually prepared one day prior to being used for the first time in an experiment, using the vehicle considered most appropriate.

Insoluble compounds were subjected to 15-30 min. treatment in a sonifying water bath, warmed to 45°C, vortexed, and used as a suspension if a full solution was not achieved. Each was distributed to sterile injection bottles, sealed and stored at 4°C until used. During use, each was stored at room temperature unless we were advised to the contrary. 1-ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin, AVS01) was included in each series of experiments as a known positive control.

Experiment Design: A total of 10 s.c.-infected mice were treated with each drug dosage, and 20 infected mice were treated with placebo (drug vehicle) as virus controls. Five sham-infected mice were used in each drug dosage as toxicity controls, and 5 or 10 additional mice were used as normal controls. The toxicity and normal controls were held in a room separate from the infected area. Treatments were s.c., b.i.d. x 5 beginning 4 hr pre-virus inoculation unless another treatment schedule was recommended to us by the COTR or other individual acquainted with the material to be tested. Because of the pretreatments, the animals could not be randomized after virus infection, but the infection was given to each cage on a random, scattered basis in an attempt to randomize between cages. The animals were examined daily for death through day 21. Toxicity and normal controls were weighed on day 0 and again 18 hr after final drug treatment to ascertain weight loss or failure to gain weight. Dosages ranged in 2-fold dilutions, the number of dosages depending on the compound and what was initially known about it. A single dose of ribavirin was run in parallel as a positive control. The anti-PTV activity of this compound was described previously by us (1).

In follow-up studies to confirm initial antiviral activity seen, or when oral therapy was employed, the infection parameters were extended to include reduction in hepatic icterus (liver score assigned a reading of 0, or normal, to 4, or maximum discoloration), serum glutamic oxaloacetic and pyruvic acid transaminases (SGOT, SGPT), recoverable virus from liver and from serum of infected animals 3 or 4 days after virus inoculation. Titration of SGOT and SGPT was accomplished by using colorimetric kits from Sigma Chemical Co. (St. Louis, MO). Spectrophotometric readings for these colorimetric assays were performed in duplicate by using a microplate autoreader (EL309, Bio-Tek Instruments, Inc., Winooski, UT). Livers were homogenized to a 10% (wt/vol) suspension prepared in minimum essential medium (MEM); liver homogenates and serum samples were assayed for PTV by diluting each 10-fold to a titer of 10^{-5}; 0.2 ml of each dilution were added to triplicate cups of LLC-MK_2 cell monolayers in 96-well microplates. Viral CPE was determined after 5 days incubation at 37°C, and 50% endpoints determined.

Statistical Evaluations: Increases in survivors were analyzed using chi-square analysis with Yates' correction. Increases in mean survival times of mice that died on or before day 21 and reductions in SGOT, SGPT and PTV levels in liver or serum were evaluated using Student's *t* test. Ranked sum analysis (Wilcoxon test) was used to compare inhibition of mean liver scores.

Results and Discussion

A total of 882 experiments were run during this 5-year report period, with 121 AVS compounds being evaluated against the PTV infection.

AVS compounds considered to be non-immunomodulators which were significantly inhibitory to the PTV infection are summarized in Table IV-1. Included among these compounds was ribavirin as well as 6 compounds chemically related to ribavirin. All were either found to be orally effective or had not yet been tested orally.

Compounds thought to be acting through immunomodulation mechanisms which were highly active vs PTV in vivo are seen in Table IV-2. These 23 compounds appear to have one common immunological property: They all induce interferon (IFN), which is known to have a profound effect on PTV (3, 7, 1990 Annual Report). With one compound, AVS5587, which both induces IFN and activates natural killer cells, pretreatment with anti-IFN antibody completely eliminated the usual anti-PTV effects of this compound (7, 1990 Annual Report). Probably most effective of all these immunomodulators were poly IC•LC (AVS1761), ampligen (AVS2149), and a poorly defined poly IC•LC derivative (AVS5593). Only bropirimine (AVS2776), CL246,738 (AVS1968) and AM-5 (AVS4282) had efficacy when given orally (by gavage) to the infected animals. All active substances exerted their antiviral effects therapeutically, i.e., after virus inoculation. None were effective if treatment began later then 48 hr after the virus, however,

which is not surprising, for by this time the disease has progressed rapidly in the animals (see Section VIII of the Report for a full description of the disease).

Non-immunomodulating AVS compounds considered slightly or moderately inhibitory to the PTV infection are seen in Table IV-3. These compounds represent a rather broad range of chemical substances, of which only a few are ribavirin derivatives. In some cases, relatively high therapeutic indices (TI) are noted, but often this was seen at a single dose; higher doses, while tolerated in the mouse, yielded no antiviral effect.

Table IV-4 summarizes the immunomodulating substances having slight to moderate inhibition to the PTV disease. Again, if an erratic dose response was seen, it was so noted, but in our view reduced the potential usefulness of the compound.

Those AVS compounds not shown to inhibit the hepatotropic PTV infection are seen in Table IV-5. In many cases, only one or two tests were run, with the compound nontoxic at all doses run, suggesting they may need to be resynthesized and further tests performed.

Conclusions

A total of 882 experiments were run in evaluating 121 AVS compounds against the hepatotropic PTV infection. Ribavirin (AVS01) and six chemical derivatives were considered markedly effective and acting specifically against the virus infection. A total of 23 immunomodulating substances also had strong anti-PTV effects. An apparent common immunological property among the latter PTV inhibitors was the induction of IFN by each compound.

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Table IV-1. AVS Non-Immunomodulating Compounds Considered Significantly Inhibitory to Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVSNo.	Compound Name	Maximum Therapeutic Indices[a] Survivor Increase	Reduction in Liver Score	Reduction in SGOT	Reduction in SGPT	Liver Virus Inhibition	Serum Virus Inhibition	Orally Active?
01	Ribavirin	65	65	200	200	200	200	yes
02	Ribavirin triacetate	57	65	65	200	65	65	yes
111	Tiazofurin	16	65	65	65	8	8	yes
206	Ribamidine	65	65	200	200	65	200	yes
253	Selenazofurin	6	12	6	6	3	3	yes
257	Tiazofurin 5'-MP	≥5[b]	≥5	≥5	≥5	≥5	≥5	?[c]
3706	Tiazofurin triacetate	≥9	≥3	≥18	≥36	≥18	≥18	?

[a]LD50 dose ÷ minimum effective dose.

[b]"≥" for therapeutic indices indicates an LD50 dose was not achieved.

[c]"?" indicates no oral test was run.

Table IV-2. AVS Immunomodulating Compounds Considered Significantly Inhibitory to Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVSNo.	Compound Name	Maximum Therapeutic Indices[a] Survivor Increase	Reduction in Liver Score	Reduction in SGOT	Reduction in SGPT	Liver Virus Inhibition	Serum Virus Inhibition	Orally Active?	Active Therapeut?[b]
1754	MVE-2	≥16[c]	≥16	≥16	≥16	≥8	≥16	No	48 hr post
1761	Poly IC•LC	1000	100	1000	1000	3125	3125	No	48 hr post
1767	AM-3	125	1	≥40	≥40	≥40	≥40	No	48 hr post
1778	Mannozym	≥129	≥65	≥129	≥129	≥1	≥1	No	48 hr post
1968	CL246,968	≥32	≥4	≥8	≥8	≥8	≥8	Yes	24 hr post
2149	Ampligen	1000	100	100	100	100	100	No	48 hr post
2776	Bropirimine	20	20	40	40	40	20	Yes	48 hr post
2779	MVE-1	≥275	≥138	≥138	≥138	≥275	≥138	No	24 hr post
3588	Meta fluorobropirimine	16	nd	nd	nd	nd	nd	?d	24 hr post
4282	AM-5	≥10	≥10	≥10	≥10	≥10	≥10	Yes	24 hr post
4286	P-136	≥65	nd	nd	nd	nd	nd	?	≥24 hr post
4287	P-117	190	190	190	190	190	190	?	≥24 hr post
5311	Human rIFN	≥100	≥32	≥320	≥320	≥320	≥320	?	24 hr post
5587	7-thia-8-oxoguanosine	26	26	26	52	26	13	?	36 hr post
5588	"ICLC"	100	313	100	100	100	100	?	≥4 hr post
5589	"ICL-CMA"	40	40	40	40	40	40	?	≥4 hr post
5590	"ICL-CMD"	313	313	100	100	313	313	?	≥4 hr post
5591	"ICL-CMB-C-dextrin"	313	1000	313	313	3125	3125	?	≥4 hr post
5592	"ICL-GEL"	313	313	313	313	313	313	?	≥4 hr post
5593	"ICL-sulfated gel"	1000	1000	1000	313	313	313	?	≥4 hr post
5594	"ICL-(PLL-dextran)"	100	1000	100	100	40	40	?	≥4 hr post
5595	"IC-(PLL-dextran)"	100	100	100	100	100	100	?	≥4 hr post

[a]LD50 dose ÷ minimum effective dose.

[b]Times shown are the latest that treatment could be initiated and significant antiviral activity achieved. "≥" indicates that time was the last time initiated; it is possible the material would still be active if therapy was started later.

[c]"≥" for therapeutic indices indicates an LD50 dose was not achieved.

[d]"?" indicates no oral test was run.

Table IV-3. AVS Non-Immunomodulating Compounds Considered Slightly or Moderately Inhibitory to Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVS No.	Compound Name	Maximum Therapeutic Index—Any Parameter	Active Orally?	Comments
52	Thioformycin B	≥24 (MST)	Yes	
65	Formycin B	16 (Survivors only)	?	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	24 (MST)	Yes	Erratic–best when given 48 hr post
215	3-Deazaguanosine	16 (MST)	?	Best when given i.p.
272	3-Deazaguanine	27 (liver score only)	Yes	
347	Phyllanthoside	3 (survivors, SGOT)	?	
1212	Uridine 2',3'-dialdehyde	8 (MST, SGOT, SGPT)	?	Erratic, not always dose-responsive
1976	Thymineriboside 2',3'-dialdehyde	6 (MST only)	?	
2700	6-Ethylthiopurine riboside	~12 (survivors, MST, SGOT, SGPT)	Yes	Erratic, not always dose-responsive
2811	7-Deoxynarciclasine	≥4 (survivors)	?	Active only at one dose, insuff. to retest
2812	Narciclasine	8 (MST, liver score, SGOT, SGPT, virus titers)	?	Highly treatment schedule dependent
2885	3-T-Butyl-1-adamantylthiourea	~32 (liver score, SGOT, SGPT, liver virus)	?	Erratic—not dose dependent
3425	8-Bromoguanosine	~4.5 (survivors, MST)	No	Erratic—not dose dependent, active given in 1 shot only
3580	Unidentified	~16 (MST only)	?	Active in 1 shot 24 hr pre only
4272	Unidentified	~16 (all parameters)	?	Erratic—not dose responsive, active s.c. only
4617	Glycine derivative of ribamidine	~10 (MST)	?	Treatment with higher doses needed

Table IV-4. AVS Immunomodulating Compounds Considered Slightly or Moderately Inhibitory to Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVS No.	Compound Name	Maximum Therapeutic Index—Paramter Used For TI Determination	Active Orally?	Comments
1969	CL 259,763	100 (liver score, SGOT, SGPT)	Yes	
2276	Theracel #BL-002	~5 (SGOT, SGPT, virus titers)	Yes	Active only at a single mid-range dose
2285	Theracel #BL-012	~31(SGOT, SGPT, virus titers only)	Yes	Pretreatment most effective
2777	AIPP	4 (liver score, SGOT, SGPT, virus titers)	Yes	Higher doses prevented death
2778	ABMP	8 (liver score, SGOT, SGPT, virus titers)	Yes	Somewhat erratic dose response
2880	Oxamisole	8 (MST)	Yes	Higher doses prevented death; somewhat erratic dose response
3587	ACPP	15 (SGOT, SGPT)	Yes	Higher doses prevented death
3589	CFABPP	2 (liver score, SGOT, SGPT, virus titers)	Yes	
3593	LY253,963	52 (MST)	Yes	Highly erratic dose response
3925	duPont A2222-1	≥8 (survivors)	?	Active at low doses only
3926	duPont A2227-1	~4 (survivors)	?	Highly erratic dose response
3927	duPont A754-1	1 (survivors)	?	Active prophylactically only
3934	Germanium, Ge132	~16 (survivors, liver score, SGOT, SGPT, virus titers)	Yes	Highly erratic dose response
4283	AM-6	≥16 (MST)	?	Higher doses prevented death
4284	AM-7	≥16 (MST)	?	
4285	AM-8	~4 (survivors)	?	Most active therapeutically, insuff. for retesting
4593	P-188	~16-32 (survivors)	?	Most active therapeutically, insuff. for retesting
5079	hu Recomb. IL-2	~16 (SGOT, SGPT, virus titers)	?	Higher doses prevented death
5596	Heat-cycled ICLC	≥1 (liver score, SGOT, SGPT, virus titers)	?	

Table IV-5. AVS Compounds Considered Inactive Against Hepatotropic Punta Toro Virus Infections in Mice.

Compound AVS No.	Compound Name	Comments
147	Enviroxime	Single i.p. treatment 4 hr post prevented death at high dose; this has not been confirmed
167	Glycerrhetic Acid	Only s.c. bid, tid x 5 regimens used.
212	Suramin	6 separate treatment regimens studied
222	3-Bromo-4-chloropyrazolo-[3,4-d]pyrimidine	6 separate treatment regimens studied
233	Formycin	1 dose caused significant increases in MST
360	7-Deoxynarciclasin	1 mid-range dose caused significant increase in MST in only 1 experiment run—nontoxic at all doses
361	Pancratistatin	Only 1 expt. run—nontoxic at all doses
1757	Isoprinosine	Only 1 expt. run, using manufacturer's recommended regimen
1777	Streptonigrin	6 separate treatment regimens studied
1976	Thymine riboside 2',3'-dialdehyde	Singe i.p. treatments caused occasoinal MST increases
2713	Bryostatin 2	2 Treatment regimens studied—nontoxic at all doses
2716	Unidentified	Only 1 test run—nontoxic at all doses
2741	Ribavirin tetrahydropyrimidine	Only 2 tests run—nontoxic at all doses
2742	Ribavirin-5-OH-tetrahydropyrimidine	3 separate treatment regimens used—nontoxic all all doses
2786	Unidentified	Only 1 test run—nontoxic at all doses
2978	Tetraacetate ester of 2980	1 mid-range dose caused survivor increase. Not yet confirmed
2980	Tetrahydroxy analog of Pancratistatin	4 separate treatment regimens studied
3585	Neurotropin	4 separate treatment regimens studied
3679	1-(4-Methoxybenzyloxy)adenosine	Only 1 expt. run—all doses nontoxic
3933	Ge089	Only 1 expt. run—all doses nontoxic
3960	DMG	4 separate treatment regimens studied
4113	Pseudolycorine HCl	MST increase at lowest dose only. Only 1 expt. run to date
4206	3-Acetamido-7-amino-6-methyl-7H-5-triazolo[5,1-C]-S-triazole	Marginal effects seen at highest dose, which was nontoxic
4273	2,3-Dihydro-5-iodothiophene-1,1-dione	5 separate treatment regimens studied
4588	1-Aminoadenosinium mesitylenesulfonate	Nontoxic at all doses used
4616	Noxymethylpennicillinic acid	3 separate treatment regimens studied—nontoxic at al doses
4618	5'-N,N-diethylthiocarbamate-5'-deoxy-5'-thioadenosine	
5027	Imexon	2 regimens which showed efficacy against a retrovirus infection were used

V. EFFECT OF AVS COMPOUNDS ON NEUROTROPIC INFECTIONS INDUCED BY THE BALLIET STRAIN OF PUNTA TORO VIRUS

Introduction

It has been stressed from the inception of this project that the PTV infection in mice is being used as a model for Rift Valley fever and sandfly fever infections in man. A late and often fatal form of Rift Valley fever involves encephalitis, and patients with sandfly fever also develop certain symptoms suggestive of central nervous system (CNS) infection. We therefore felt it was important to determine if AVS compounds active against the hepatotropic Adames PTV infection would also have an effect on an encephalitic disease induced in mice by the neurotropic (Balliet) strain of PTV. As described earlier, our protocol for *in vivo* evaluations of anti-PTV compounds includes follow-up testing of PTV-inhibitory compounds against the CNS disease in mice. The results of these follow-up investigations are described in this section.

Materials and Methods

Virus: The Balliet strain of PTV was obtained from the American Type Culture Collection (ATCC, Rockville, MD). This virus was originally isolated from a young adult male in Panama in 1966. The virus was twice plaque purified through LLC-MK_2 cells, and a pool subsequently made in these cells. Virus identity was confirmed by serum neutralization.

Animals: Four week-old female Balb/c or Swiss Webster mice were obtained from Simonsen Laboratories. The animals were quarantined 48 hr prior to use and were maintained on standard mouse chow and water *ad libitum*. The Swiss Webster mice were used when i.v. administration of drugs was given.

Compounds: All compounds were initially provided by Technassociates, Inc. and later by Biological Research Faculty and Facility, Inc. (Rockville, MD). Compounds synthesized by Pharmatek, Inc. were especially prepared using dimethylsulfoxide per chemist's instructions.

Experiment Design: Ether-anesthetized mice were infected by inoculating 0.05 ml of PTV i.c. into the right hemisphere of the brain. Twenty infected mice were used with each drug level, with 5 infected mice used as virus controls which received drug diluent only. Treatment and schedule varied depending upon the compound being evaluated, with those regimens considered highly effective against the hepatotropic virus infection selected for treatment of this CNS disease. Five toxicity control mice were used at each drug dose level, and 10 mice were used as normal controls. The latter two groups of controls were weighed before and after treatment as described earlier. On infection day 6, one-half (one or two pre-designated cages) of each group of infected animals were killed and their brains removed. Ten percent homogenates of each brain were diluted through a series of 10-fold dilutions and each was assayed for virus using CPE production in triplicate cups of LLC-MK_2 cells. The remaining animals were observed daily for death through infection day 21, which was the termination of the experiment.

Increases in survivor number were evaluated using chi square analysis with Yates' correction. Increases in mean survival time and decreases in mean brain virus titers were analyzed using *t* test.

Results and Discussion

Compounds considered to have exerted some degree of inhibition to the neurologic disease are summarized in Table V-1. No AVS compound was considered to be strikingly effective against this infection. Some compounds prepared by Pharmatek, Inc. (AVS 5896, 6080, 6082) to particularly penetrate the lipophilic blood brain barrier were also only marginally effective.

Compounds considered inactive against this virus infection are summarized in Table V-2. Most of the Pharmatek AVS01 derivatives were included in this group. Most of the Pharmatek materials were administered i.v. or i.v. and i.p. on alternating days using dosages designed to approach the maximum tolerated as determined by preliminary toxicity determinations.

Some experiments utilized the virus administrated by intranasal (i.n.) administration in an attempt to reduce the initial severity of the infection and thus afford the test compound a better opportunity to exert a positive antiviral effect. Several compounds (AVS01, 206, 2776) used in both i.n. and i.c.-infected mice were essentially equally marginally effective. Since the i.n.

administration route usually was not uniformly lethal to the mice, we discontinued using that method of virus exposure.

It was thought that interferon (IFN) inducers such as AVS1761 and 2149, which are markedly effective against the hepatotropic disease in mice, would also prove useful against this infection; AVS2149 was moderately inhibitory to the infection, but AVS1761 displayed no inhibitory effects in this infection model.

These data indicate a real need for compounds which can more effectively control the neurotropic PTV infection.

Conclusions

A total of 51 AVS substances were evaluated in one or more experiments against the fatal neurologic infection induced by the Balliet strain of PTV in mice. Thirteen compounds (AVS01, 02, 206, 253, 1754, 2149, 2776, 3588, 3589, 3934, 5896, 6080, 6082) exerted some degree of inhibition of this infection, but none were considered markedly active.

Table V-1. AVS Compounds Considered Inhibitory[a] to Neurotropic Punta Toro Virus Infections in Mice

Compound AVS No.	Name	Therapeutic Index: Survivor Increase	Therapeutic Index: MST Increase	Therapeutic Index: Brain Virus Decrease	Comments
01	Ribavirin	0	2	0	Treatment i.p. or i.v.
02	Ribavirin triacetate	0	4	2	
206	Ribamidine	0	1	2	Once only i.p. therapy
253	Selenazofurin	0	8	0	Not dose-responsive
1754	MVE-2	0	2	0	
2149	Ampligen	8	4	16	4 tests run with differing regimens active in 2 tests
2776	Bropirimine	0	8	4	Brain virus reduction study done in intranasally inoculated virus, repeated with i.c. inoculated virus
3588	Metafluoro ABPP	0	0	1,8	Erratic dose response
3589	5-Chloro-2,3-difluorophenyl ABPP	0	0	1	
3934	Ge132	0	0	1	
5896	Pharmatec 01 derivative	0	2	0	i.v. + i.p. therapy
6080	Pharmatec 01 derivative	0	≥4	0	erratic dose response
6082	Pharmatec 01 derivative	≥2	≥2	≥2	

[a]Rendered a statistically significant improvement in any parameter.

Table V-2. AVS Compounds Considered Inactive Against Neurotropic Punta Toro Virus Infections in Mice

Compound AVS No.	Name	Comments
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	2 Treatment regimens used
111	Tiazofurin	
215	3-Deazaguanosine	
272	3-Deazaguanine	
1018	Phenyleneamine	Reduced brain virus titer at low dose only
1761	Poly IC•LC	
1767	AM-3	
1778	Mannozym	
1968	CL246,738	
1968	CL259,763	
2700	6-Ethyl thiopurine riboside	
2777	AIPP	
2779	MVE-1	
2880	Oxamisole	2 Treatment regimens used—some activity seen at erratic doses
2933	CGP19835A lipid	3 Treatment regimens used
3706	Tiazofurin triacetate	
4282	AM-5	3 Treatment regimens used
5054	Pharmatec 01 derivative	Administered i.v. in DMSO
5055	Pharmatec 01 derivative	Administered i.v. in DMSO
5056	Pharmatec 01 derivative	Administered i.v. in DMSO
5057	Pharmatec 01 derivative	Administered i.v. in DMSO
5221	Pharmatec 01 derivative	Administered i.v. in DMSO
5222	Pharmatec 01 derivative	Administered i.v. in DMSO
5311	Human recombinant interferon	
5581	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
5582	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
5786	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
5897	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
5898	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6081	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6083	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6290	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6291	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6292	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6297	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6300	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6417	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO
6477	Pharmatec 01 derivative	Administered i.v. + i.p. in DMSO

VI. EFFECTS OF DRUG COMBINATIONS ON THE HEPATROPIC PUNTA TORO VIRUS INFECTION IN MICE

Introduction

It is a recognized concept that the prudent use of two or more drugs in combination will often result in an improved effect against certain diseases when compared to using the drugs by themselves. An objective in this contract research work was to examine certain PTV disease inhibitors in various combinations in an attempt to ascertain those which may have clinical potential.

Two approaches were generally made in these experiments; the first utilized an experiment design oriented to determine if the drug combination had an increased therapeutic index (TI) compared to using either drug alone. Such combinations would conceivably reduce the risk of toxicity when treating the disease because less drug would be required to yield a positive therapeutic effect. The second approach was to determine if the use of one drug, such as a recognized immunomodulator, would significantly reduce the toxicity of high dosages of another, more standard, antiviral drug. Thus an "antidote" for the better drug could potentially be developed. In the latter approach, we concentrated our efforts primarily in attempting to reduce the toxicity of ribavirin (AVS01).

To orient these experiments to apply as much as possible to clinical situations, oral therapy was used where feasible, and initiation of treatments was after virus inoculation.

Materials and Methods

Virus: The Adames strain of PTV as described earlier was used. The virus concentration was selected to be approximately 95% lethal to the mice used.

Animals: Female 3 week-old C57BL/6 mice weighing 10-13 g were obtained from Simonsen Laboratories (Gilroy, CA). Quarantine, caging, and feeding of these mice was as described in Section IV.

Compounds: All compounds were initially provided by Technassociates, Inc., and later by Biological Research Faculty and Facility, Inc. (Rockville, MD). The following drug combinations were studied:

AVS01 (ribavirin) + AVS219 (ampligen)

AVS01 + AVS1754 (MVE-2)

AVS01 + AVS2776 (bropirimine)

AVS01 + AVS2779 (MVE-1)

AVS01 + AVS5587 (7-thia-8-oxoguanosine)

AVS01 + AVS1761 (poly IC•LC)

AVS206 (ribamidine) + AVS2776

AVS206 + AVS1767 (AM-3)

Each drug was prepared in the vehicle considered most appropriate; for AVS01 and AVS206, this was sterile water for injection. AVS2149 was first annealed according to manufacturer's directions, then diluted in physiological saline for these studies. AVS1761, 1754, 1767, and 2779 were dissolved in physiological saline. AVS2776, which is quite water-insoluble, utilized 0.4% carboxymethylcellulose (CMC).

Experiment Design: Treatment regimens for each drug combination are summarized in Table VI-1. In these studies, 5 to 6 experiments were run in parallel, according to the following general scheme:

#1: Compound A (AVS01 or 206) at 4 or 5 dosages. These dosages in some experiments included a usually lethally toxic dose and 3 or 4 usually marginally PTV-active or -inactive dosages.

#2: Compound B (always an immune modulator) at three doses ranging from active to inactive against PTV.

#3: Compound A at all dosages used in #1 + Compound B used at the highest dose only.

#4: Compound A at all dosages used in #1 + Compound B used at the mid dose only.

#5: Compound A at all dosages used in #1 + Compound B used at the lowest dose only.

An expanded parameter anti-PTV experiment as described in Section IV was run in each study, the disease parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus and serum virus titer, with 20 infected mice used in each treatment group, 40 mice used as placebo-treated controls, 10 mice as normal controls, and 5 animals in each treatment group as toxicity controls. One-half of each treatment group, virus controls, and normal controls were killed 4 days after virus inoculation, bled, and their livers removed. Livers were scored from 0 to 4, homogenates prepared from each, and the homogenates tested for virus titer; serum was assayed for SGOT, SGPT, and PTV titers. The remainder of the mice were held 21 days post-virus inoculation with deaths noted daily.

Statistical Evaluations: Alterations in the various virus parameters were analyzed by the standard statistical tests described in Section IV. Determinations of antagonistic, additive, or synergistic drug interactions were made by calculating fractional inhibitory concentration (FIC) indices, as was described by Berenbaum (1). In this method, the FIC was determined using the modified formula:

$$FIC = \frac{\text{MIC of Drug A in Combination}}{\text{MIC of Drug A alone}} + \frac{\text{MIC of Drug B in Combination}}{\text{MIC of Drug B alone}}$$

This FIC has been used by Huggins et al. (2) and Allen et al. (3) in their combination studies. All et al. (3) has interpreted the FIC values as:

FIC < 0.5: Significant synergism
FIC 0.5 - 0.9: Suggestive of synergism
FIC ~1: Effects are additive
FIC 1.1-1.9: Indifference or partial antagonism
FIC ≥2: Antagonism

In certain experiments as appropriate, the graphic method of Huggins et al. (2) was also used, with the location of the line of calculated ED50 values relative to the expected line indicating whether the drug combination was synergistic, additive, or antagonistic.

Results and Discussion

A summary of all combination experiments run is seen in Table VI-2. Of the nine drug combination studied, four were considered synergistic, one additive to slightly synergistic, three were antagonistic, and one yielded insufficient data. Each individual combination will be discussed in the following:

Combination #1: AVS01 + AVS2149: This was one of the earliest experiments run by us, and all doses of AVS2149 were markedly effective, Hence, FIC indices, which use MIC values, could not be calculated. For details see our 1987 Annual Report.

Combination #2: AVS01 and AVS2149: this is somewhat of a repeat of #1, but AVS2149 was given in only a single injection. In this study, strong synergy was seen using all parameters with this drug combination. Analyzing these data from a TI standpoint, in which TI = maximum tolerated dose (MTD) divided by minimum inhibitory concentration (MIC), the results were as follows:

AVS01 used alone: TI = ~120
AVS2149 used alone: TI = ~100
AVS01 + AVS2149 (0.005 mg/kg): TI = 600

These data indicate the drug combination was more effective than either drug used alone. It should be pointed out that an exact MIC for AVS01 was not obtained in this study; 10 mg/kg/day showed marginal antiviral activity using one parameter, so this dose was assumed to be the MIC. Also, an MTD was not reached for AVS2149. For the purposes of calculating the data shown above, 5 mg/kg was used, which, while tolerated, caused some weight loss.

Of considerable importance in considering this drug combination is the reduction in AVS01 toxicity seen by also treating the mice with AVS2149 (Table VI-3). All doses of AVS2149 reduced the lethal toxicity of ribavirin.

As a drug combination to render greater antiviral efficacy, AVS01 + AVS2149 appears appropriate. AVS01 (ribavirin) has a multiplicity of antiviral actions, all oriented toward biochemically blocking viral synthesis in the cell (review, 4). AVS2149 (ampligen) renders its anti-PTV effect presumably because it is a rapid inducer of high IFN levels in the serum (5). It is known that PTV is highly sensitive to IFN (6, 1989 Annual Report). Thus this combination employs radically different approaches to preventing viral synthesis.

The lessening of toxicity is more difficult to explain; it is known that ribavirin at high doses is immunosuppressive (review, 4), but it is not known whether AVS2149 acts to reverse that immunosuppression to reduce ribavirin's toxicity or if some other mechanism is involved.

The timing of AVS2149 treatment may be most meaningful; this compound was given 1 hr prior to start of AVS01 therapy. Whether AVS2149 treatment beginning after AVS01 will also render a reduced toxicological effect remains to be determined.

For further detail on this experiment, see our 1990 Annual Report.

Combination #3: AVS01 + AVS1754: In this study, survivor, liver score and SGOT parameters indicated strong synergy, but SGPT, liver virus and serum virus indicated additive to antagonistic effects. The mean of all the FIC indices, 0.93, was indicative of additive to slightly synergistic effects. Again, this is a combination of a specifically antiviral agent (AVS01, ribavirin) with an immunomodulator (AVS1754, MVE-2) with a spectrum of immunological effects. These effects include activation of peritoneal macrophages, stimulation of natural killer (NK) cells, T cells and B cells (review, 7). We have shown the compound to induce moderate amounts of IFN (1989 Annual Report).

This experiment is described extensively in our 1988 Annual Report.

Combination #4: AVS01 + AVS2776: Strong synergy was seen using this combination at all parameters for evaluation. The combination employs ribavirin with bropirimine, which has a spectrum of immune stimulatory effects, including macrophage activation, augmentation of NK cell cytotoxicity, interleukin-1 and interleukin-2 stimulation, enhancement of antigen-mediated antibody formation, stimulation of bone marrow proliferation, and, most importantly, rapid induction of high levels of interferon (review, 8, and data reported by us in our 1989 Annual Report).

The details of this experiment are described in our 1989 Annual Report.

There has been a suggestion that AVS2776 may also reverse ribavirin-induced lethal toxicity, but repeated studies by us yield highly erratic results, so this conclusion cannot yet be substantiated.

Combination #5: AVS01 + AVS2779: Using all evaluation parameters, this combination using the protocol indicated was considered slightly to strongly antagonistic. This combination employs ribavirin with MVE-1, a lower molecular weight polymer related to MVE-2 and with most of the same immunological profile (7). In our experiments with PTV, MVE-1 has had essentially the same activity as has MVE-2. It is interesting that, although used in an identical manner as MVE-2 (see combination #3), MVE-1 was antagonistic when used with ribavirin while MVE-2 appeared somewhat synergistic. This difference in efficacy may have something to do with the ability of each polymer to persist in the animal, rendering a prolonged immunologic effect. See the 1989 Annual Report for details of this experiment.

Combination #6: AVS01 + AVS5587: Using all parameters but survivors, the combination was considered strongly synergistic in its action against the PTV infection. The survival data were somewhat adversely affected because 25% of the placebo-treated virus controls survived in the experiment. This combination utilizes ribavirin with 7-thia-8-oxoguanosine, the latter being a nucleoside analog with the immunologic properties of rapidly inducing IFN and activating NK cells (9-11, 1990 Annual Report). We have shown that the IFN-inducing properties of this compound are the primary PTV-inhibitory effects (12, 1990 Annual Report). The unique 2-injection schedule

utilized for AVS5587 was derived from our previous studies which indicated this treatment regimen was the most effective when used vs PTV.

A significant property of AVS5587 is that it reduced ribavirin's lethal toxicity, as summarized in Table VI-4. This table shows the data from the above-cited combination study as well as other data further investigating this phenomenon.

See also the 1990 Annual Report for these experiments.

Combination #7: AVS01 + AVS1761: This combination appeared initially to be a logical follow through of combinations #1 and 2, since AVS1761 is poly IC•LC, a polynucleotide related to ampligen and which is also a potent IFN inducer. A difference in this study compared to the earlier combination treatments with AVS2149 is that AVS1761 was administered on an every other day treatment schedule. This may be significant, since this drug combination was considered rather strongly antagonistic using every evaluation parameter. It should also be noted that AVS1761 therapy was initiated the same time as AVS01, instead of 1 hr pre as used in combination #2. In view of these rather conflicting results, we recommend further studies be run with this combination.

This experiment was described in our 1990 Annual Report.

Combination #8: AVS206 + AVS2776: This drug combination utilized ribamidine, a chemical derivative of ribavirin, with bropirimine which was described in combination #4, above. Another difference in this study compared to combination #4 is that AVS2776 was given once only, compared to 3 separate treatments in the previously cited study. The data in this experiment were rather unusual, in that AVS206 when used alone, exerted strong anti-PTV effects at low dosages usually found ineffective. When run in combination with AVS2776, the various disease parameters generally showed a lesser effect than when AVS206 was used alone. Hence, the experiment appears to be strongly antagonistic. We recommend this experiment be repeated.

See the 1988 Annual Report for further details on this study.

Combination #9: AVS206 + AVS1767: This experiment combined therapies with the ribavirin analog ribamidine used with AM-3, also known as inmunoferon, a fungal product developed by Spanish investigators. The material enhances IFN induction (13), NK cell activity (13, 14), lymphocyte proliferation (14), interleukin-2 response (14), T-cell mitogen response (13, 14), and to restore antibody responses and delayed hypersensitivity reactions (15). We suspect the primary mechanism of PTV inhibition is a result of AM-3's IFN induction properties. This combination was considered to be strongly synergistic in its action (Table VI-2).

See the 1988 Annual Report for other details on this drug combination.

Conclusions

A total of 9 drug combinations were evaluated against the hepatotropic PTV infection in vivo. The combinations of AVS01 + AVS2149, AVS01 + AVS2776, AVS01 + AVS5587, and AVS206 + AVS1767 were considered synergistic in their action. AVS01 + AVS1754 had additive or slightly synergistic effects. The combinations of AVS01 + AVS2779 and AVS01 + AVS1761 were considered antagonistic in their action. AVS206 + AVS2776 appeared antagonistic, but due to some questionable data will be repeated.

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Table VI-1. Drug Combinations Studied in the Hepatotropic Punta Toro Virus Model.

Combination #	Compound AVS No.	Treatment Route	Beginning of Treatment	Treatment Schedule
1	01 +	p.o.	+24 hr	bid x 5
	2149	i.p.	+24 hr	qd x 5
2	01 +	p.o.	+24 hr	bid x 3
	2149	i.p.	+23 hr	once only
3	01 +	p.o.	+24 hr	bid x 5
	1754	i.p.	+24 hr	once only
4	01 +	p.o.	+24 hr	bid x 5
	2776	p.o.	+24 hr	qd x 3
5	01 +	p.o.	+24 hr	bid x 5
	2779	i.p.	+24 hr	once only
6	01 +	p.o.	+24 hr	bid x 3
	5587	i.p.	+24, +31 hr	bid x 1
7	01 +	p.o.	+24 hr	bid x 3
	1761	i.p.	+24 hr	eod x 2
8	206 +	p.o.	+24 hr	bid x 5
	2776	p.o.	+24 hr	once only
9	206 +	p.o.	+18 hr	bid x 5
	1767	s.c.	+48 hr	once only

Table VI-2. Values for the Various Drug Combinations Evaluated Against the Hepatotropic Punta Toro Virus Infection

Drug Combination No. (AVS Nos.)	Evaluation Parameter	FIC Index	Mean FIC	Interpretation
1 (01 + 2149)	all	*		insuff. data
2 (01 + 2149)	survivors	0.28		
	liver score	0.18		
	SGOT	0.35		
	SGPT	0.35		
	liver virus	0.35		
	serum virus	0.35		
			0.31	Synergistic
3 (01 + 1754)	survivors	0.15		
	liver score	0.2		
	SGOT	0.13		
	SGPT	2.0		
	liver virus	2.0		
	serum virus	1.1		
			0.93	Additive to sl. synergistic
4 (01 + 2776)	survivors	≤0.6		
	liver score	≤0.6		
	SGOT	≤0.6		
	SGPT	≤0.6		
	liver virus	≤0.6		
	serum virus	≤0.6		
			0.6	Synergistic
5 (01 + 2779)	survivors	1.3		
	liver score	3.0		
	SGOT	2.1		
	SGPT	3.0		
	liver virus	2.0		
	serum virus	1.5		
			2.2	Antagonistic
6 (01 + 5587)	survivors	1.7		
	liver score	0.6		
	SGOT	0.6		
	SGPT	0.4		
	liver virus	0.2		
	serum virus	0.04		
			0.6	Synergistic
7 (01 + 1761)	survivors	4.2		
	liver score	2.0		
	SGOT	4.2		
	SGPT	3.3		
	liver virus	6.4		
	serum virus	6.4		
			4.4	Antagonistic

Drug Combination No. (AVS Nos.)	Evaluation Parameter	FIC Index	Mean FIC	Interpretation
8 (206 + 2776)	survivors	0.8		
	liver score	34.3		
	SGOT	17.9		
	SGPT	33.3		
	liver virus	0.7		
	serum virus	0.5		
			14.6	Antagonistic [?]**
9 (206 + 1767)	survivors	0.7		
	liver score	0.1		
	SGOT	0.3		
	SGPT	0.3		
	liver virus	0.3		
	serum virus	0.8		
			0.13	Synergistic

*2149 was highly effective at all dosages, so FIC indices could not be determined.

**Questionable due to highly erratic indices. AVS206 used alone was much more effective than usual.

Table VI-3. Reduction of AVS01-Induced Murine Toxicity by AVS2149 Therapy[a]

Compound (AVS No.)	Dose (mg/kg/day)	% Survivors
01	1500	0
2149	0.005 to 5	100
01 + 2149	1500 + 5	60**
01 + 2149	1500 + 0.5	40*
01 + 2149	1500 + 0.05	60*
01 + 2149	1500 + 0.005	100*

[a]AVS01: p.o. bid x 3; AVS2149: i.p., one injection 1 hr pre-AVS01

*P<0.05 **P<0.01 compared to AVS01 used alone.

Table VI-4. Reduction of AVS01-Induced Murine Toxicity by AVS5587 Therapy[a]

Compound (AVS No.)	Dose (mg/kg/day)	% Survivors	Comments
Expt. 1 (male mice)			
01	1250	0	
5587	6.25	100	
	12.5	100	
	25	100	
01 + 5587	1250 + 6.25	0	
	1250 + 12.5	0	
	1250 + 25	80**	
Expt. 2 (male mice)			
01	1750	40	
	1500	20	
	1200	20	
5587	25	100	
01 + 5587	1750 + 25	80	5587 given on 3rd
	1500 + 25	80*	day of 01 therapy
	1250 + 25	100**	
01 + 5587	1750 + 25	60	5587 given on 4th day
	1500 + 25	60*	of 01 therapy
	1250 + 25	60*	
01 + 5587	1750 + 25	20	5587 given on 5th day
	1500 + 25	0	of 01 therapy
	1250 + 25	80*	
Expt. 3 (female mice)			
01	1750	40	
	1500	0	
	1250	80	
5587	25	100	
01 + 5587	1750 + 25	40	5587 given on 3rd day
	1500 + 25	20	of 01 therapy
	1250 + 25	80	
01 + 5587	1750 + 25	20	5587 given on 4th day
	1500 + 25	80**	of 01 therapy
	1250 + 25	40	
01 + 5587	1750 + 25	40	5587 given on 5th day
	1500 + 25	100**	of 01 therapy
	1250 + 25	80	

[a]Expt. 1: AVS01: p.o. bid x 3; AVS5587: i.p., 0 and 7 hr relative to AVS01. Expts. 2 and 3: AVS01: p.o. bid x 5; AVS5587: i.p., single injection at times indicated.

*$P<0.05$ **$P<0.01$ compared to AVS01 used alone.

VII. COMPARISONS OF THE ANTI-PUNTA TORO VIRUS EFFICACY OF AVS01, AVS02, AND AVS206

Introduction

Three related compounds, AVS01 (ribavirin), AVS02 (ribavirin triacetate), and AVS206 (ribamidine) have exerted strong in vitro and in vivo activity against PTV. A series of carefully controlled experiments were run comparing the anti-PTV efficacy of each compound.

This section summarizes the results of these extensive experiments and our conclusions regarding the potential efficacy of each compound.

Table VII-1 itemizes the questions we endeavored to answer in these experiments.

Results and Discussion

Therapeutic Index Comparisons

Table VII-2 shows the experiment design for comparisons of therapeutic indices (TI) of each compound. Both s.c. and p.o. routes of treatment were examined, and expanded parameters used as criteria for evaluations, with assays taken on days 2 and 5 of the infection.

Table VII-3 summarizes the comparative LD50 toxicity of each compound administered s.c. or p.o. twice daily for 5 days. Ribavirin appeared to be considerably more toxic than either AVS02 or 206.

The relative TI's of each compound administered s.c., with viral assays done on day 2 are shown in Figures VII-1 and VII-2. AVS01 was clearly more effective in reducing SGOT and SGPT values at this early assay time (Figure VII-2). When assayed on infection day 5, however, no definitive differences were seen (Figures VII-3, 4).

Oral therapy results with assays done on day 2 are seen in Figures VII-5 and VII-6. Overall, no significant differences were seen between the three compounds. The day 5 assay results are summarized in Figures VII-7 and VII-8, with no strong differences again seen. Effects of p.o. therapy on white blood cell decline are summarized in Figure VII-9.

Effects of Delaying Treatment

Comparisons of the delay of initiation of s.c. once daily therapy with LD/4 doses of each compound are summarized in Figure VII-10. Only AVS206 was effective when therapy was delayed to 72 hr after virus inoculation. When therapy was increased to twice daily, the results were less definitive (Figure VII-11). Once daily p.o. treatment with each compound yielded essentially equal results (Figure VII-12). When p.o. treatments were given twice daily, AVS02 appeared somewhat more efficacious (Figure VII-13).

Effects of Reducing Treatment Duration

In this series of experiments, p.o. treatments given twice daily were reduced by one-day increments from 5 days to 1 day. An LD50/64 dose was used of each compound. No clear-cut differences in antiviral effect were seen between any compound, although some erraticism in results was observed (Figure VII-14). A similar experiment was run using the LD50/4 dose of each compound given s.c. As seen in Figure VII-15, no differences were seen.

Influence of Increasing Viral Challenge

These experiments utilized p.o. therapy twice daily for 5 days beginning 24 hr post-virus inoculation. Viral challenge varied by one-half $\log_{10}$ from 10^{-3} to 10^{-5} of our standard virus stock. The results are summarized in Figure VII-16. In this study, AVS206 had increased TI's over the other compounds at the lowest and highest virus challenge doses. We attribute this difference to experimental variation.

Effects on i.c.-Administered Balliet Strain PTV

Four doses of each drug, each equivalent to the other according to the LD50 data obtained earlier, were used i.p. twice daily for 5 days beginning 4 hr pre-virus inoculation against the PTV encephalitic infection. Essentially no antiviral activity was seen with any compound, although AVS01 significantly reduced brain virus titers at the highest tolerated dose without prolonging death or increasing survivors.

Effect on Daily Disease Parameters in Hepatotropic PTV Infections

In this final series of experiments, PTV-infected mice were treated orally a single time with the LD50/16 dose of each compound, and various tissues and serum taken from 6 animals killed on days 1-10, 13, 16, 19, and 22. Disease parameters included hepatic icterus, SGOT, SGPT, total WBC, and viral titers in serum, liver, mesenteric lymph node, spleen, kidney, lung, spinal cord, and brain. Mice were perfused with sterile saline prior to tissues being taken for viral assay.

These experiments were too extensive to summarize well. The following briefly describes the results using each disease parameter.

Effect on Prevention of Death: The results of treatment with these compounds on PTV-associated death in the mice are summarized in Figure VII-17. The placebo-treated virus control animals began dying on day 4 and continued dying until day 8, with a mean survival time of 5.2 days. All three compounds were essentially equally effective in preventing death. At the dosage used, all appeared well tolerated, although the toxicity control mice treated with AVS01 gained the least weight in this experiment.

Effect on Hepatic Icterus: (Figure VII-18). Liver scores developed rapidly in the virus control mice to reach peaks by 4 to 6 days after virus inoculation. Of the 3 compounds, treatment with AVS02 inhibited the development of liver scores to the greatest extent, with AVS01 being least effective.

Effect on SGOT and SGPT: (Figures VII-19, 20). The initial development of both transaminase enzyme levels in the serum coincided well with the hepatic icterus seen, with both enzymes achieving maximal levels by day 3. The levels declined immediately thereafter for 3 days before rising again to high levels by day 7, after which all the mice had died. Treatment with all three drugs was effective in preventing these levels from elevating significantly, with AVS02 again performing best in keeping the enzymes at essentially normal levels.

Effects on White Blood Cell Counts: (Figure VII-21). Total white blood cells were assayed on days 1, 2, 4, 6, 8, 10, 13, 16, 19, and 22. As has been observed previously, these cells decline by day 2 of infection in virus control mice, presumably due to destruction of the cells by the PTV, since we have previously isolated virus from these cells. This decline was lessened by all drugs, although AVS01-treated animals exhibited the most steady increase. It should be noted that the single normal control killed and assayed at each sampling time tended to exhibit considerable fluctuation, presumably due to the method of cell counting which can be somewhat inaccurate.

Effects on Serum Virus Titers: (Figure VII-22). Viremia was seen in these s.c.-inoculated mice by the first day after virus inoculation, increasing to maximal levels exceeding 6 $\log_{10}$ by day 2 and maintaining at high levels until death of the animal. A similar, rapidly developing viremia occurred in each treated group of mice, with the titers reaching the same high level, but then rapidly declining to low or undetectable levels by day 5. Only AVS01-treated mice still exhibited approximately one $\log_{10}$ of virus on days 5 and 6. It should be pointed out that the single treatments given were done orally on day 1, after virus titers were already high in the serum.

Effects on Liver Virus Titers: (Figure VII-23). The virus titers in the livers of virus control mice increased dramatically by infection day 2 and maintained at high levels until the animals had all died. The virus titers also increased in the livers despite treatment with all three drugs but after 2 days these declined rapidly to low or below detectable levels by day 5. Only AVS01-treated mice showed relatively high virus titers (1.2 $\log_{10}$) by days 5 and 6.

Effects on Spleen Virus Titers: (Figure VII-24). The spleen virus titers developed in a pattern similar to that seen in the liver in the control mice. In the drug-treated group, the virus also developed, but to a somewhat lower maximal level and then they declined rather slowly to undetectable levels by about day 10. In this study, AVS206 was somewhat more effective than the other compounds used. In mice treated with AVS01, some virus was still seen at less than 1 $\log_{10}$ titers at the 16, 19, and 22 day sampling periods.

Effects on Kidney Virus Titers: (Figure VII-25). Kidney virus was initially seen in the control mice on day 2 of the infection. The mean virus titers at this time, which were maximal then, were approximately 4 $\log_{10}$. The virus titers in all drug-treated groups were initially one-half to over 1

log_{10} lower than those in the control mice, and, after the initial peaks seen on days 2 or 3, declined to low or undetectable levels by day 5. AVS01-treated mice continued to have detectable virus in their kidneys at 4 different assay times after the titers in mice treated with the other two drugs were below the limits of detection.

Effects on Lung Virus Titers: (Figure VII-26). Lung virus titers developed in the virus control mice essentially as in the other tissues, although did not achieve the same high titers. Therapy with the 3 drugs again had a similar effect as above, with AVS01 still having slightly less efficacy.

Effects on Mesenteric Lymph Node Virus Titers: (Figure VII-27). The virus titers in the lymph node tissue developed at the same rate as other tissues but to lower titers not exceeding 2.5 log_{10}. Drug treatment lowered the titers to limits of detection, although only AVS206 kept the titers below detectable limits.

Effects on Spinal Cord Virus Titers: (Figure VII-28). We have previously found the hepatotropic PTV to penetrate brain tissues when inoculated s.c., as described in the 1989 4th Quarterly Report. It was interesting to find that the virus was also seen in the spinal cord beginning by one day after infection. This very early occurrence of virus in this tissue suggests to us that the spinal cord itself may not have been infected, but the spinal fluid surrounding the cord would be infected since the viremia in the animal occurs very early in the infection. Perfusion of the tissue would not affect the virus in the spinal fluid. Treatment with each compound reduced the recoverable virus titers, but only AVS206 kept the virus below detectable limits.

Effects on Brain Virus Titers: (Figure VII-29). Brain virus titers were seen by day 2 of the infection; this was quite surprising, since it has been assumed that if the virus developed in the brain it would probably do so rather late in the infection. These data suggest that, although the animals were perfused, there was either virus from the spinal fluid contaminating the brain tissues, or virus in the blood remaining in smaller capillaries in the brain. Treatment with all three drugs eliminated all detectable virus by day 7; again, in view of the poor penetration of neuronal tissues by these drugs, this would suggest an effect of eliminating the viremia rather than virus in the brain tissues.

Conclusions

The overall results of these extensive experiments are briefly summarized in Table VII-4. Extensive in vivo studies were run in parallel to compare the anti-PTV efficacy of AVS01, 02, and 206. Questions considered were: Which had the greater therapeutic index when given s.c. or p.o.; which was most effective when s.c. or p.o. treatment was delayed; which was most effective when s.c. or p.o. treatment duration was reduced; which was most effective p.o. when viral challenge was increased; which was most effective against the i.c. inoculated Balliet strain of PTV; which, when administered p.o., would better control daily development of hepatic icterus, virus titers in various tissues, and the usual PTV-associated decline in white blood cells? Although the results were not fully definitive, we conclude that AVS206 is marginally better than AVS01 or AVS02.

Table VII-1. Antiviral Comparisons of Ribavirin (AVS01), Ribavirin Triacetate (AVS02), and Ribamidine (AVS206)

1. Which has the greater THERAPEUTIC INDEX?
   - given s.c. bid x 5 beginning 24 hr post (expanded parameters)
   - given p.o. bid x 5 beginning 24 hr post (expanded parameters)
2. Which is most effective when TREATMENT IS DELAYED?
   - given s.c., qd x 5 24 hr pre, 0 hr, 24, 36, 72, or 96 hr post
   - given p.o., qd x 5 24 hr pre, 0 hr, 24, 36, 72, or 96 hr post
   - given s.c., bid x 5 24 hr pre, 0 hr, 24, 36, 72, or 96 hr post
   - given p.o., bid x 5 24 hr pre, 0 hr, 24, 36, 72, or 96 hr post
3. Which is most effective when TREATMENT DURATION IS REDUCED?
   - given p.o., bid x 5, x 4, x 3, x 2, x 1, one shot
   - given s.c., bid x 5, x 4, x 3, x 2, x 1, one shot
4. Which is most effective when VIRAL CHALLENGE IS INCREASED?
   - p.o., bid x 5 beginning 24 hr post
5. Which is most effective against the INTRACEREBRALLY INOCULATED BALLIET STRAIN OF PTV?
6. Which will better control the daily development of hepatic icterus, virus titers in various tissues, and the usual PTV-associated decline in white blood cells of PTV-infected mice?

Table VII-2. Experiment Design for Comparisons of Therapeutic Indices of AVS01, AVS02, and AVS206.

Infection: 10-13 g C57BL/6 mice infected s.c. with Adames Strain Punta Toro virus.

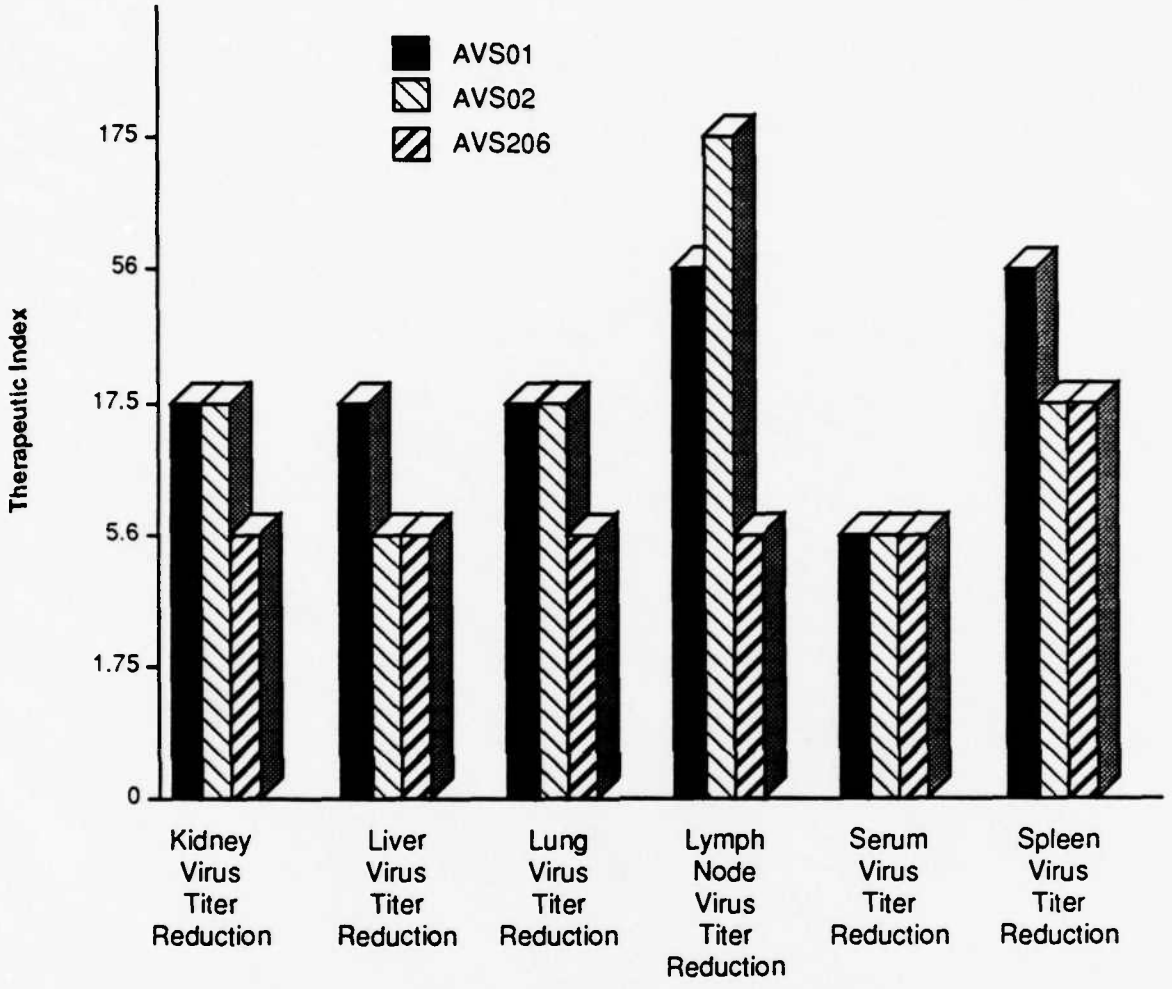
Treatment: s.c. or p.o. bid x 5 beginning 24 hr post-virus inoculation. Six dosage levels of each drug.

Evaluation: Death through 21 days. Infected, treated mice randomly selected and killed on Day 2 and on Day 5 for study of liver score, SGOT, SGPT, virus titer in liver, lung, lymph node, kidney, spleen, serum. IFN on Day 2, Total WBC on Day 5, neutralizing Ab on Day 21.

Table VII-3. Comparative LD50 Toxicity of AVS01, AVS02, and AVS206 in C57BL/6 Mice.

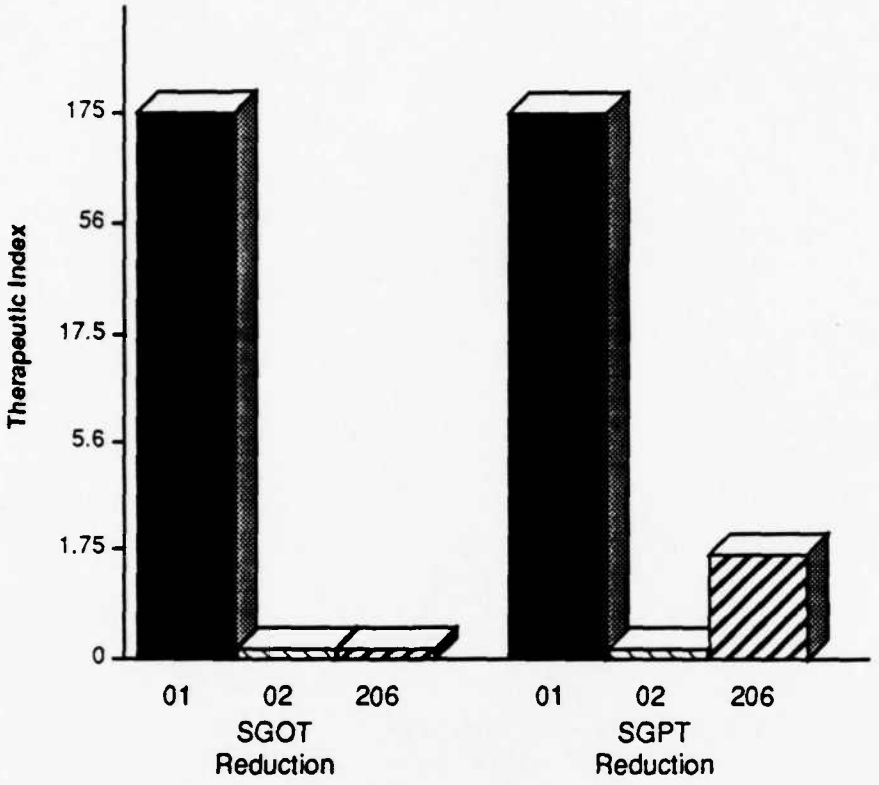
	s.c. bid x 5	p.o. bid x 5
AVS01	560	1300
AVS02	1700	2252
AVS206	1700	2600

Figure VII-1. Expts. PtA 669-671. Comparison of Virus Titer Reduction Effects of AVS01, AVS02 and AVS206 in Punta Toro Virus-Infected Mice.[a] (Assays done 2 days post-virus inoculation)



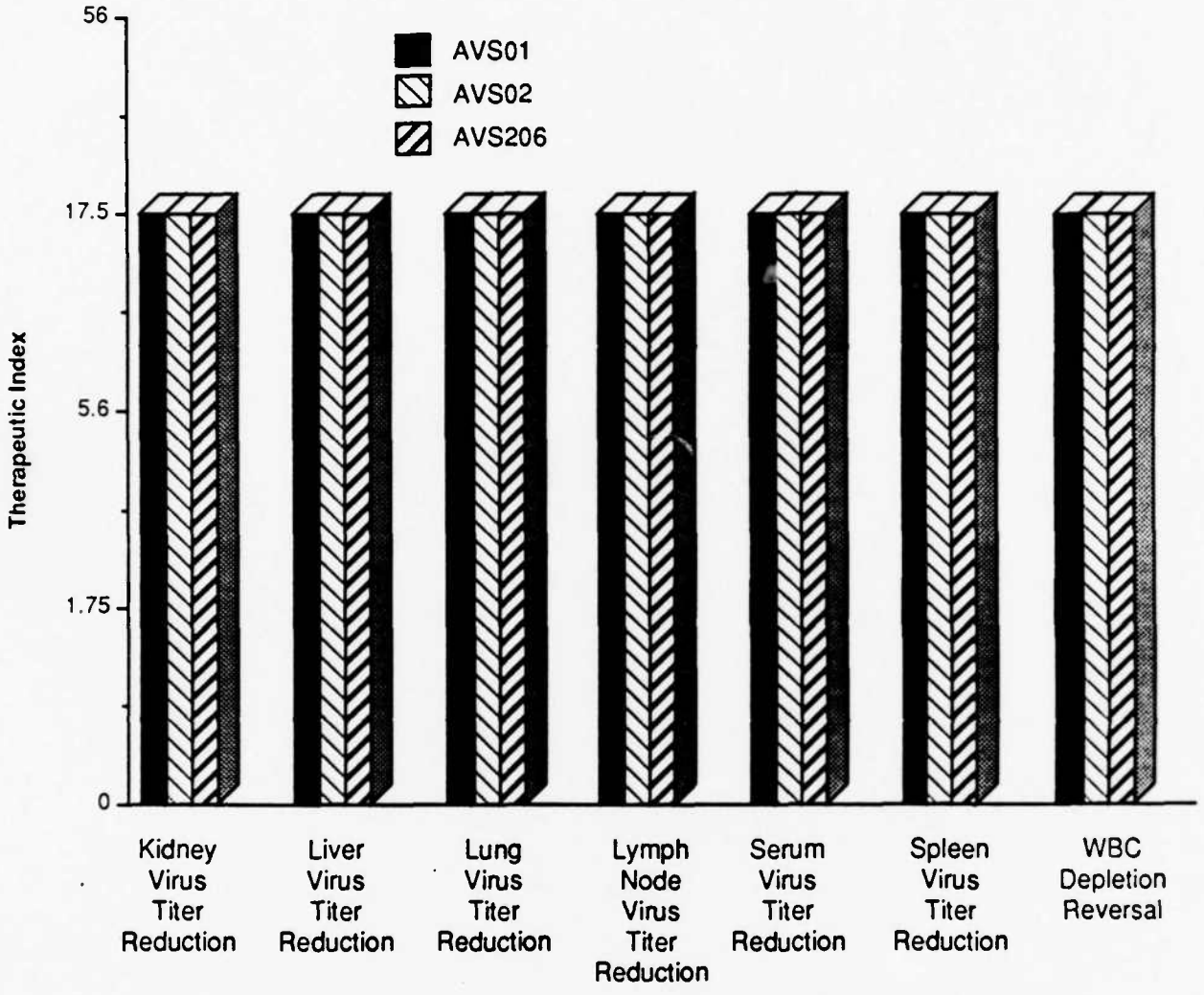
[a] Treatment s.c., bid x 5 beg. 24 hr post-virus inoculation.

Figure VII-2. Expts. PtA 669-671. Comparison of Serum Liver Enzyme (SGOT, SGPT) Reduction Effects of AVS01, AVS02 and AVS206 in Punta Toro Virus-Infected Mice.[a] (Assays done 2 days post-virus inoculation)



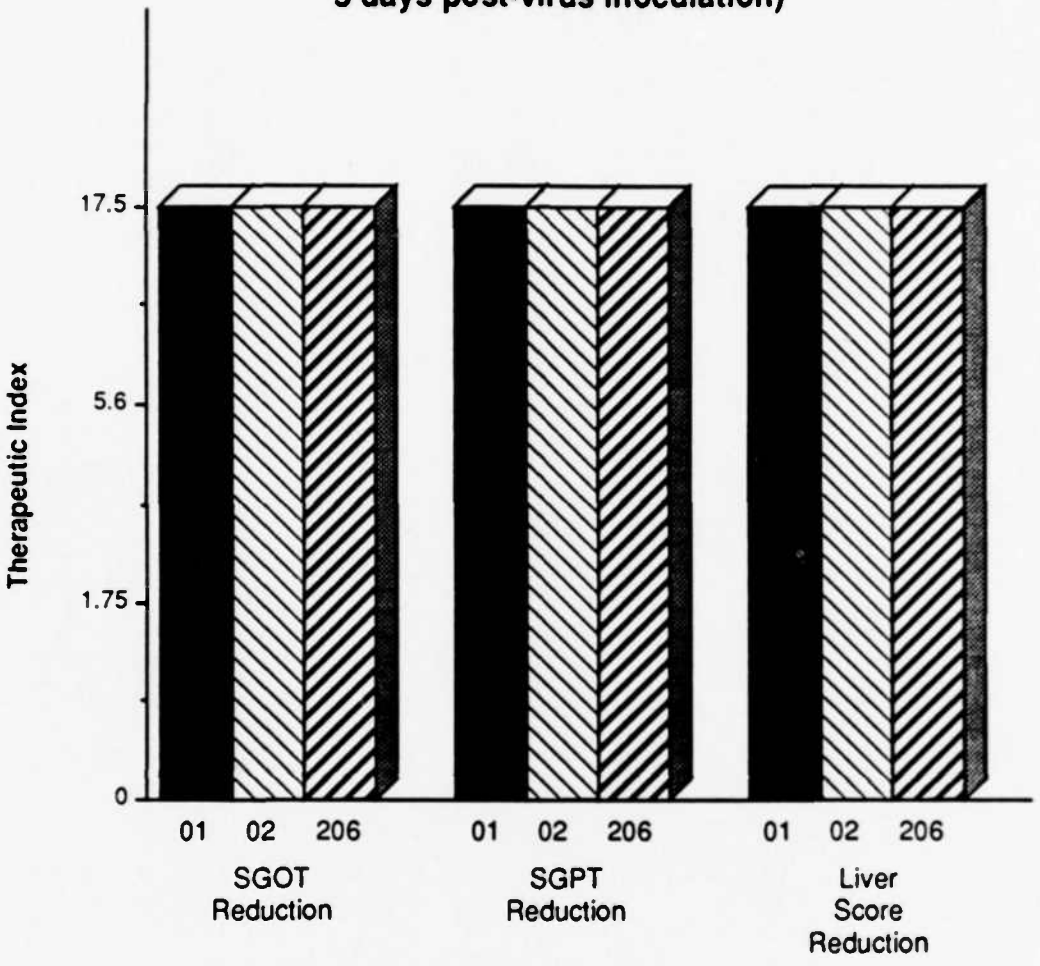
[a] Treatment s.c., bid x 5 beg. 24 hr post-virus inoculation.

Figure VII-3. Expts. PtA 669-671. Comparison of Virus Titer Reduction and Reversal of White Blood Cell Depletion Effects of AVS01, AVS02 and AVS206 in Punta Toro Virus-Infected Mice.[a] (Assays done 5 days post-virus inoculation)



[a] Treatment s.c., bid x 5 beg. 24 hr post-virus inoculation.

Figure VII-4. Expts. PtA 669-671. Comparison of Serum Liver Enzyme (SGOT, SGPT) Reduction and Liver Score Reduction Effects of AVS01, AVS02 and AVS206 in Punta Toro Virus-Infected Mice.[a] (Assays done 5 days post-virus inoculation)



[a] Treatment s.c., bid x 5 beg. 24 hr post-virus inoculation.

Figure VII-5. PtA687-689. Comparison of Virus Titer Reduction Effects of AVS01, AVS02 and AVS206 on Punta Toro Virus-Infected Mice. (Assays done 2 days post-virus inoculation)

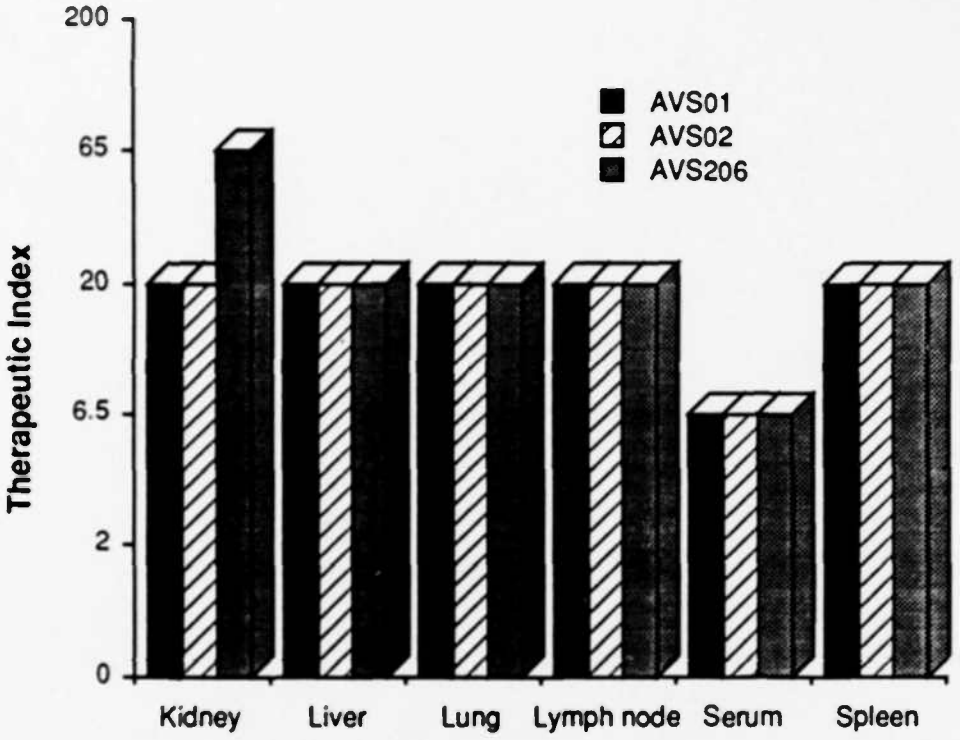


Figure VII-6. Expts. PtA687-689. Comparison of Serum Liver Enzyme (SGOT, SGPT) Reduction Effects of AVS01, AVS02, and AVS206 in Punta Toro Virus-Infected Mice. (Assays done 2 days post-virus inoculation)

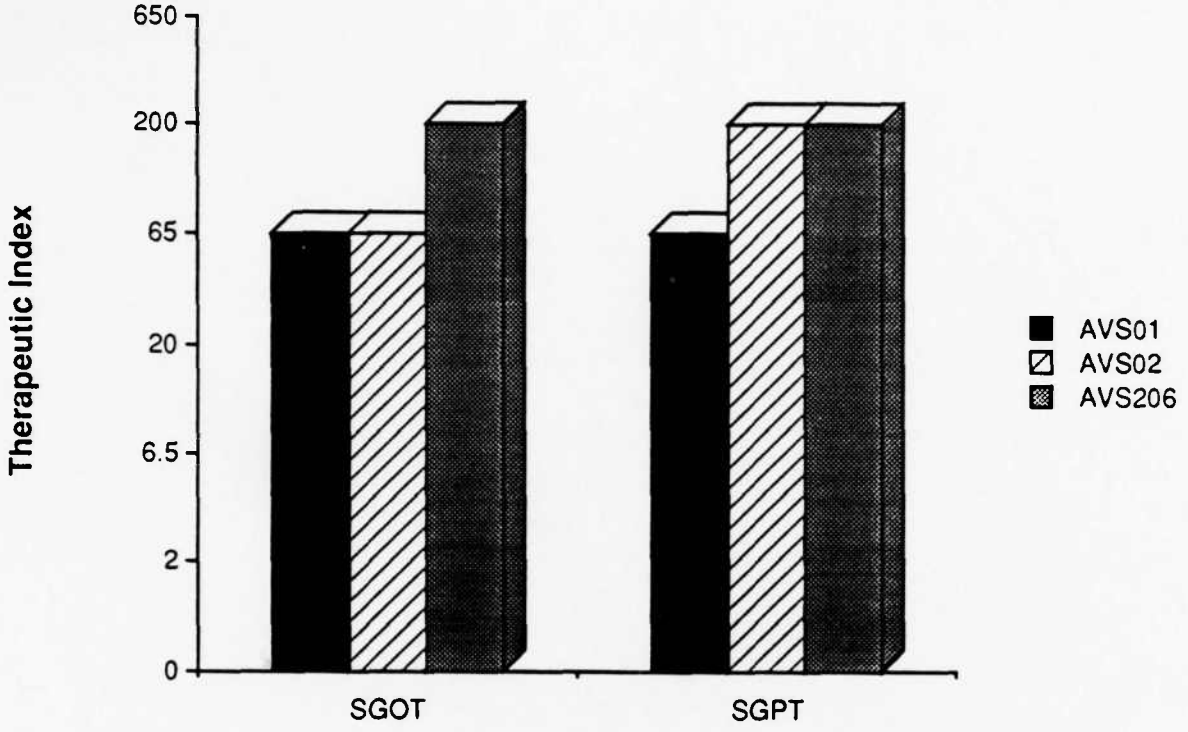


Figure VII-7. PtA687-689. Comparison of Virus Titer Reduction Effects of AVS01, AVS02 and AVS206 on Punta Toro Virus-Infected Mice. (Assays done 5 days post-virus inoculation)

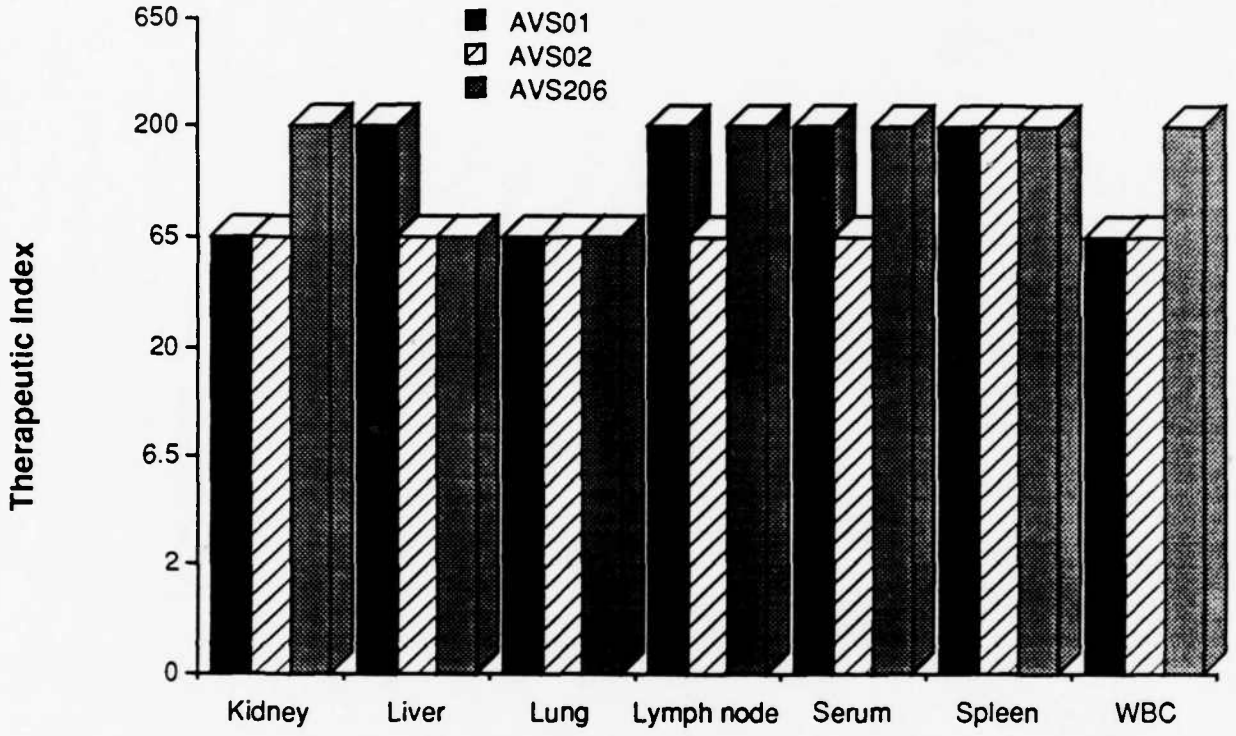


Figure VII-8. Expts. PtA687-689. Comparison of Serum Liver Enzyme (SGOT, SGPT) and Liver Score Reduction Effects of AVS01, AVS02, and AVS206 in Punta Toro Virus-Infected Mice. (Assays done 5 days post-virus inoculation)

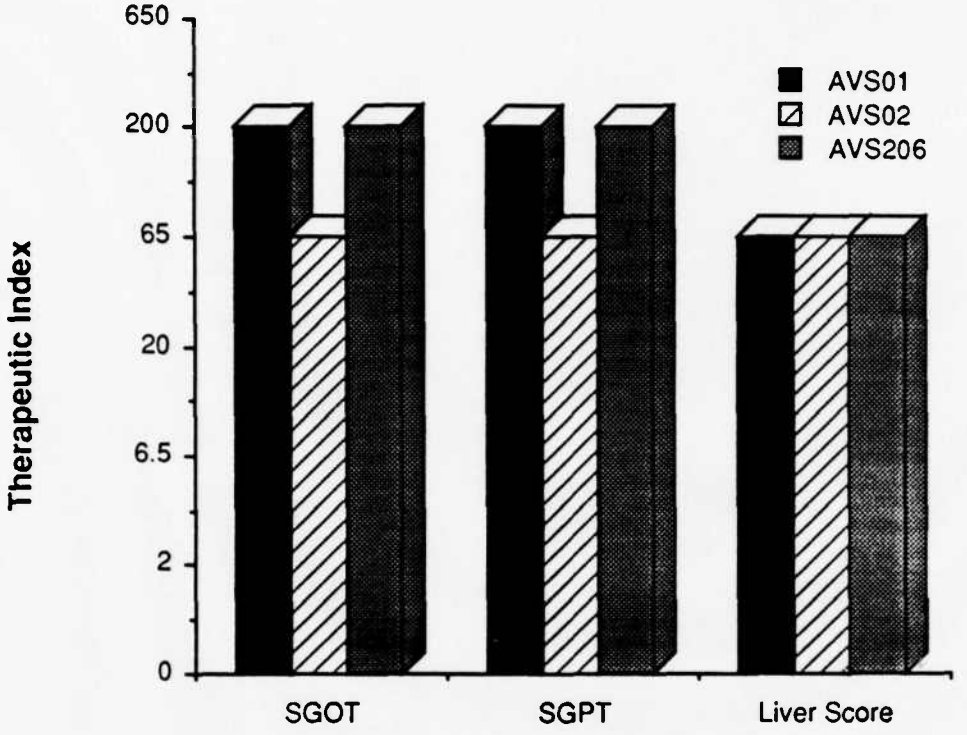


Figure VII-9. PtA687-689. Effect of p.o. Therapy with AVS01, AVS02, and AVS206 on Punta Toro Virus-Induced White Blood Cell Decline in C57BL/6 Mice. (bid x 5, beginning 24 hr post-virus inoculation)

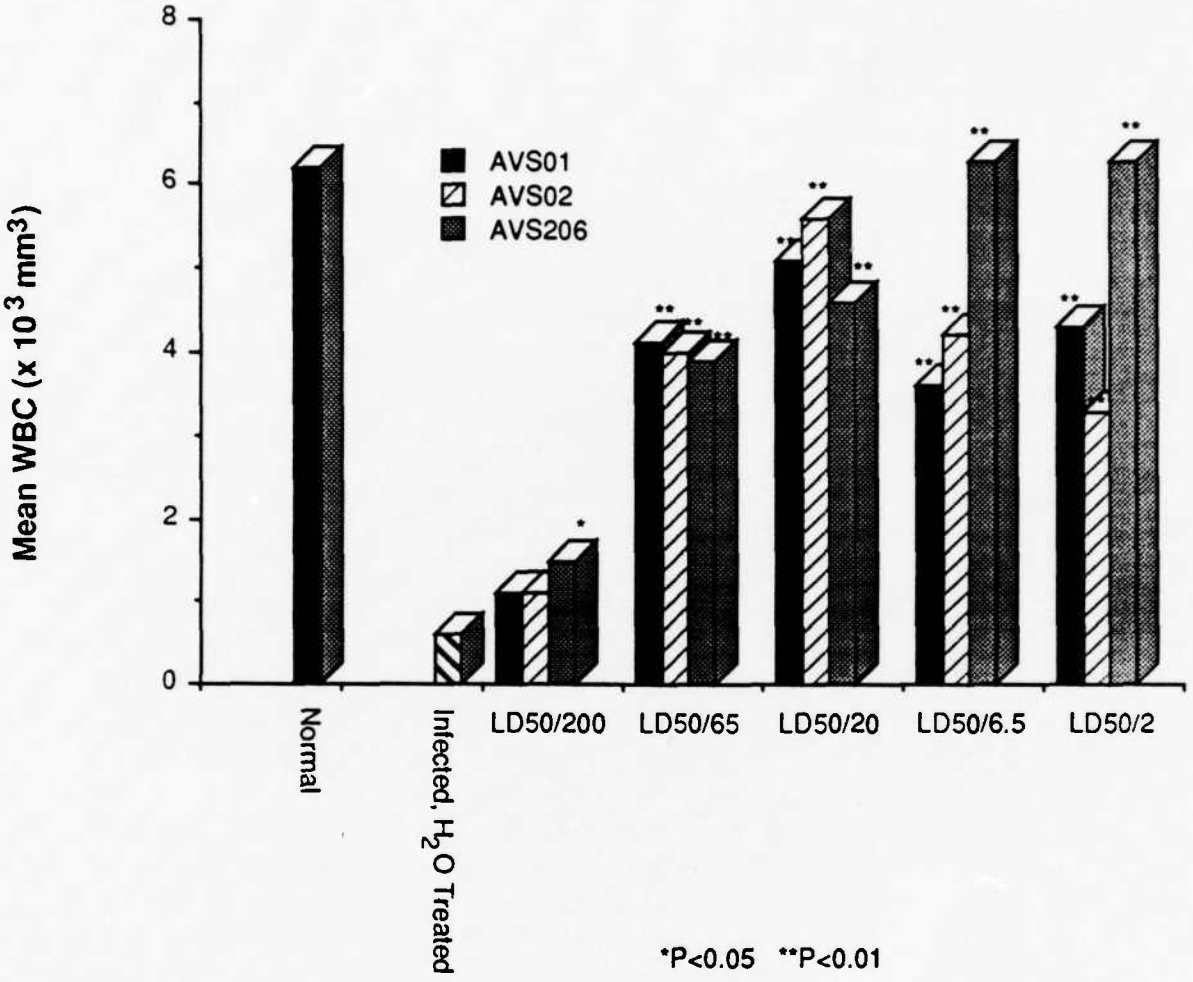
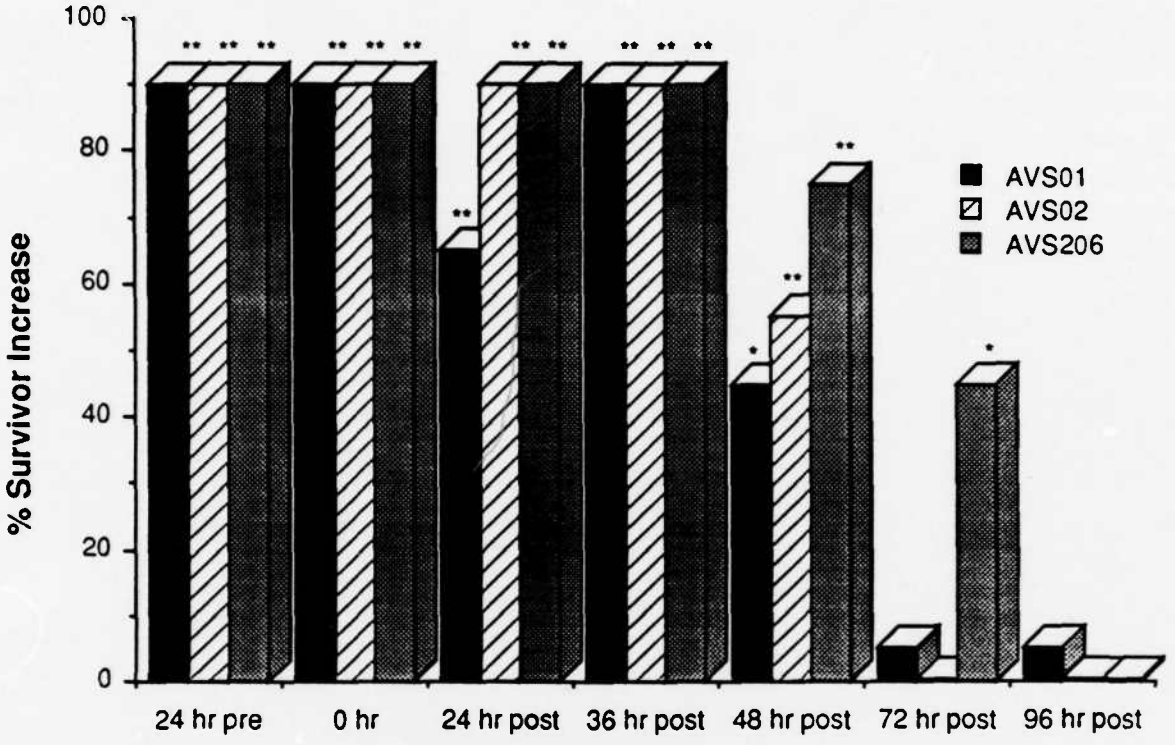
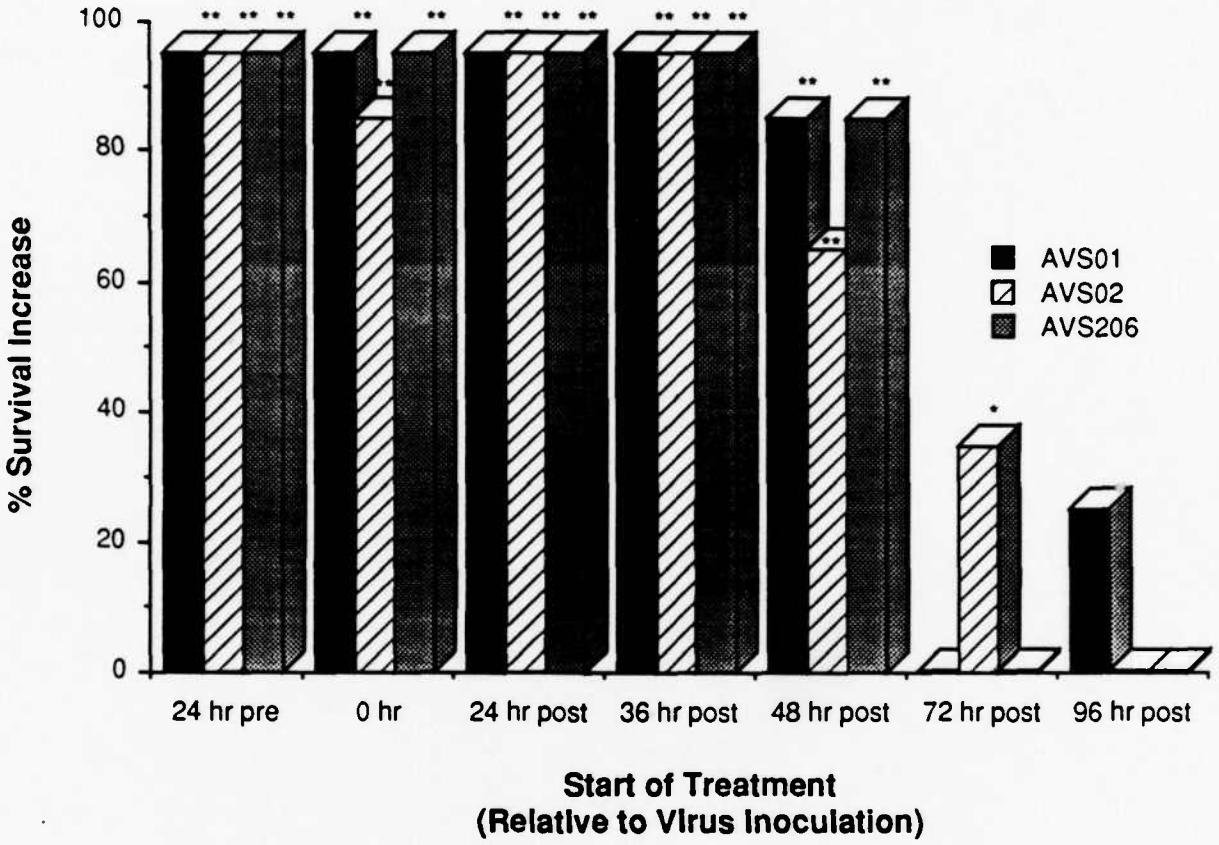


Figure VII-10. Expts. PtA690-692. Effect of Delay of s.c. qd x 5 treatment on Anti-Punta Toro Virus Activity of AVS01, AVS02, and AVS206 in C57BL/6 Mice. (LD50/4 Doses: AVS01, 140 mg/kg/day; AVS02, 425 mg/kg/day; AVS206, 425 mg/kg/day)



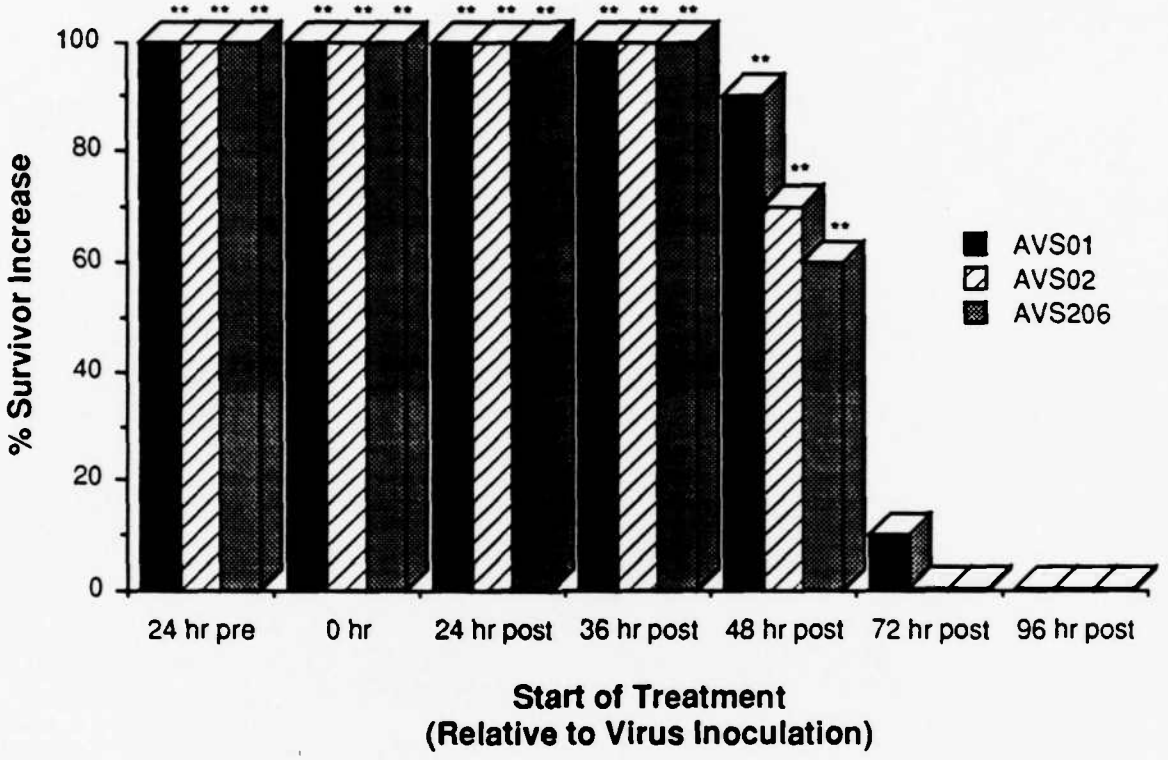
*P<0.05 **P<0.01

Figure VII-11. Expts. PtA693-695. Effect of Delay of s.c. bid x 5 treatment on Anti-Punta Toro Virus Activity of AVS01, AVS02, and AVS206 in C57BL/6 Mice. (LD50/4 Doses: AVS01, 140 mg/kg/day; AVS02, 425 mg/kg/day; AVS206, 425 mg/kg/day)



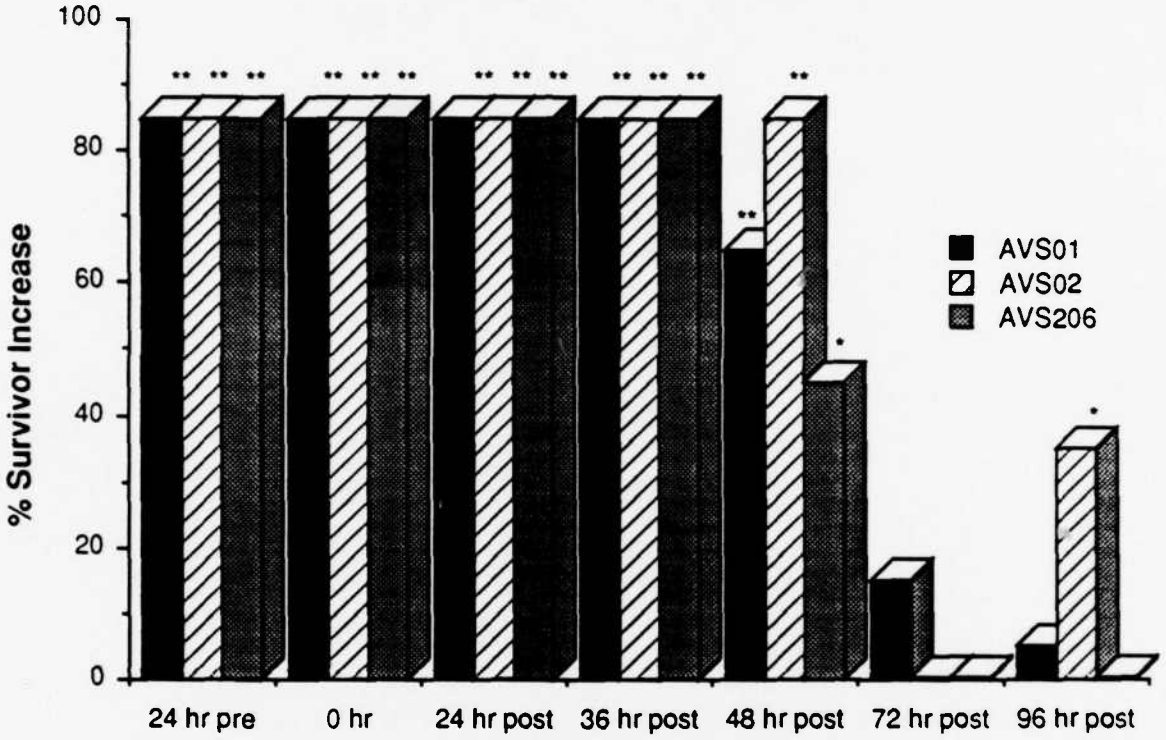
*P<0.05 **P<0.01

Figure VII-12. Expts. PtA701-703. Effect of Delay of p.o. qd x 5 treatment on Anti-Punta Toro Virus Activity of AVS01, AVS02, and AVS206 in C57BL/6 Mice. (LD50/4 Doses: AVS01, 325 mg/kg/day; AVS02, 563 mg/kg/day; AVS206, 650 mg/kg/day)



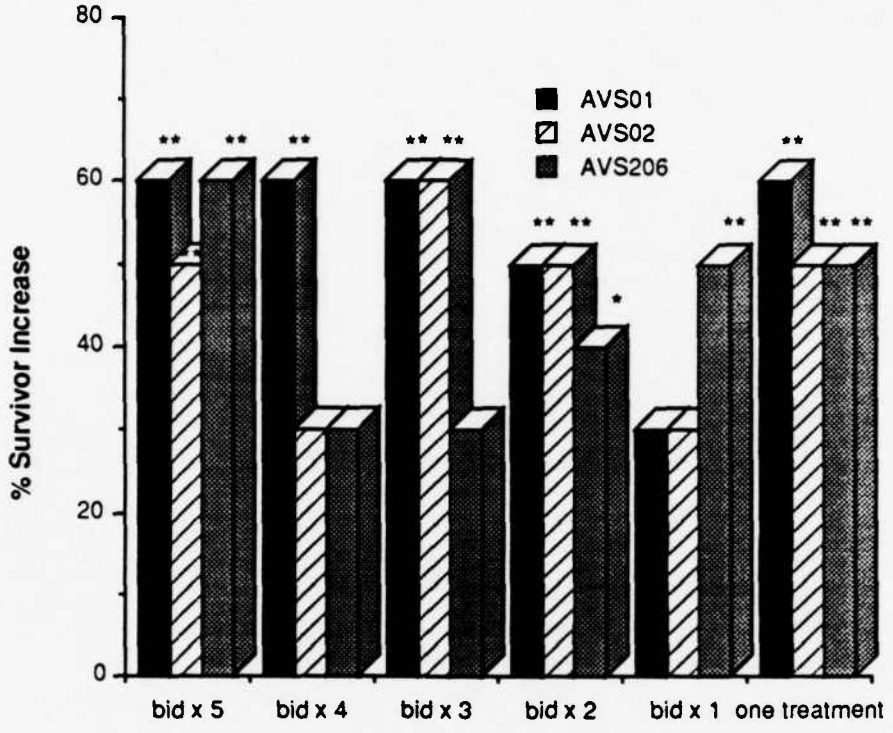
**P<0.01

Figure VII-13. Expts. PtA696-698. Effect of Delay of p.o. bid x 5 treatment on Anti-Punta Toro Virus Activity of AVS01, AVS02, and AVS206 in C57BL/6 Mice. (LD50/4 Doses: AVS01, 325 mg/kg/day; AVS02, 563 mg/kg/day; AVS206, 650 mg/kg/day)



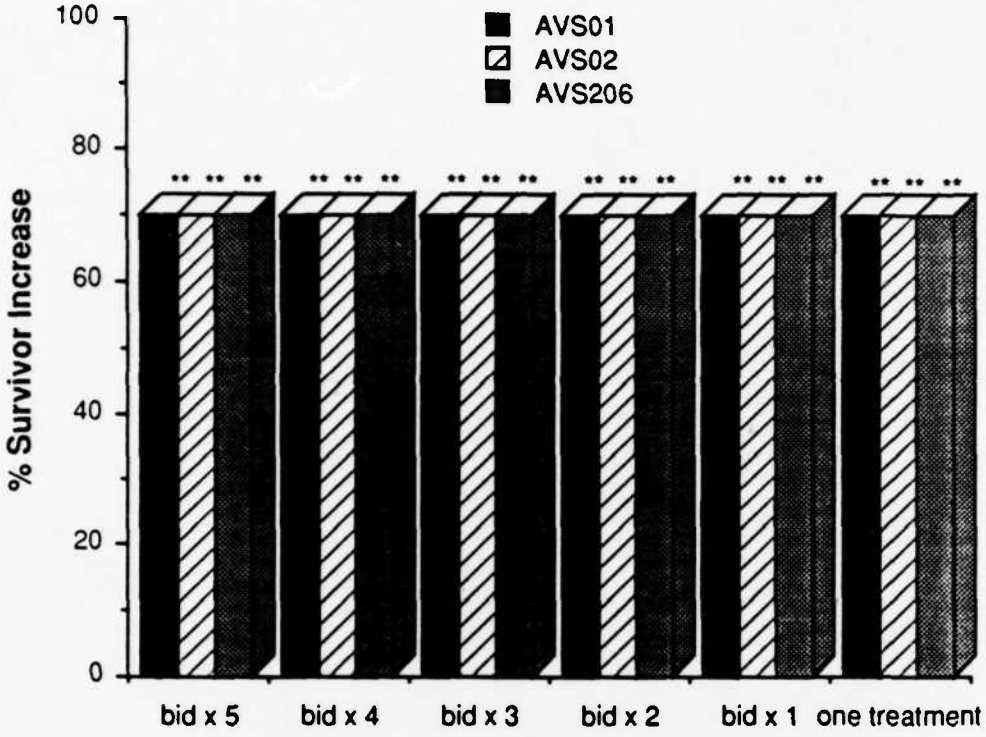
*P<0.05 **P<0.01

Figure VII-14. Expts. PtA 765-770. Effect of Reduction of p.o. Treatment Duration on Anti-Punta Toro Virus Activity of AVS01, AVS02, and AVS206 in C57BL/6 Mice. (LD50/64 Doses: AVS01, 20 mg/kg/day; AVS02, 35 mg/kg/day; AVS206, 41 mg/kg/day)



*P<0.05 **P<0.01

Figure VII-15. Expts. PtA712-717. Effect of Reduction of s.c. Treatment Duration on Anti-Punta Toro Virus Activity of AVS01, AVS02, and AVS206 in C57BL/6 Mice. (LD50/4 Doses: AVS01, 140 mg/kg/day; AVS02, 425 mg/kg/day; AVS206, 425 mg/kg/day)



**P<0.01

Figure VII-16. Expts. PtA719-733. Effect of Virus Challenge on the Anti-Punta Toro Virus Activity of AVS01, AVS02, and AVS206, Expressed as Relative Therapeutic Indices

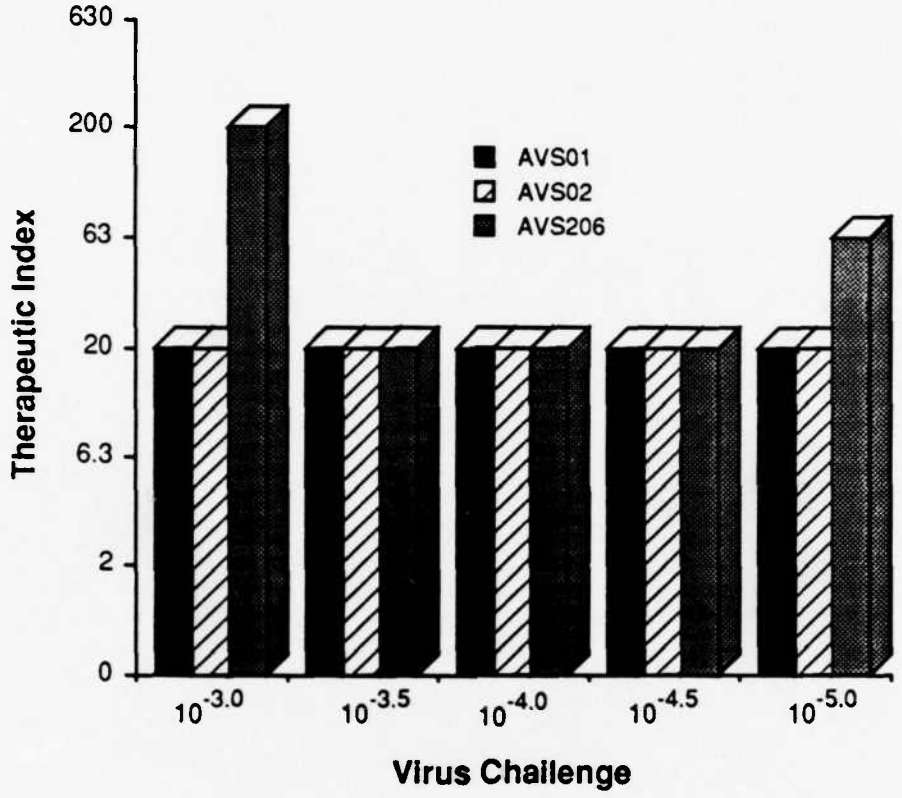


Figure VII-17. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Mean Survival Times in Punta Toro Virus-Infected Mice.

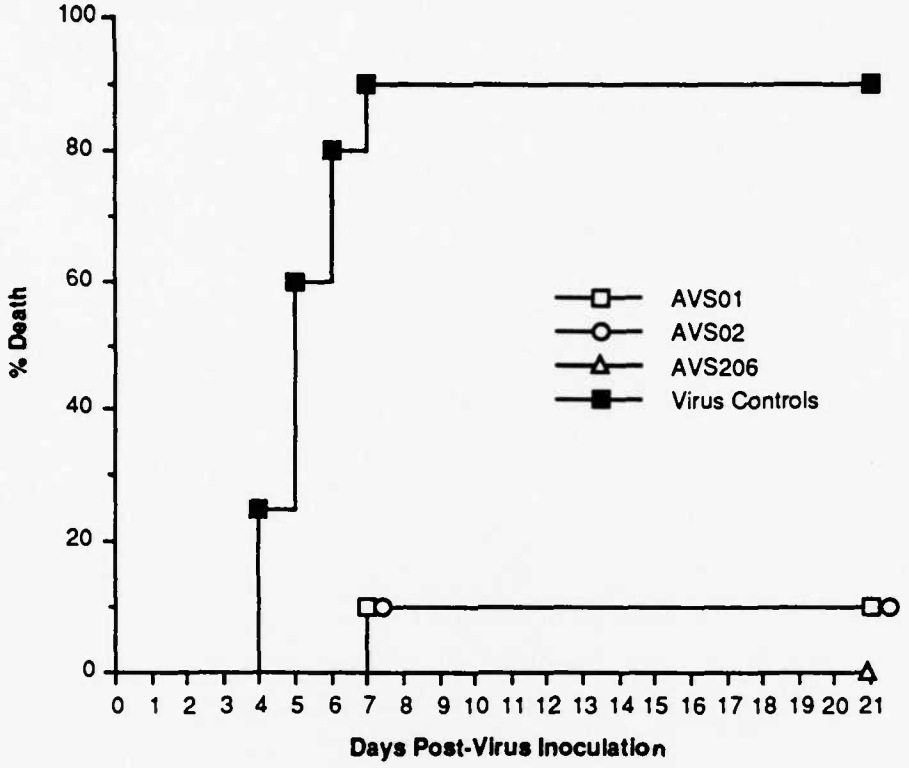


Figure VII-18. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Hepatic Icterus in Punta Toro Virus-Infected Mice.

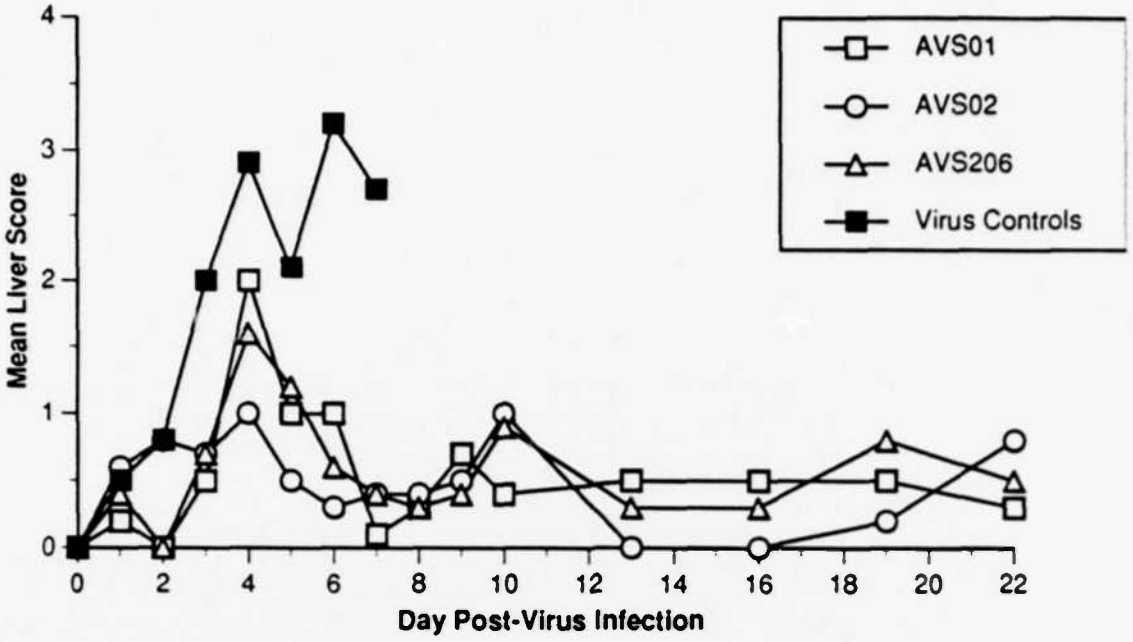


Figure VII-19. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on SGOT in Punta Toro Virus-Infected Mice.

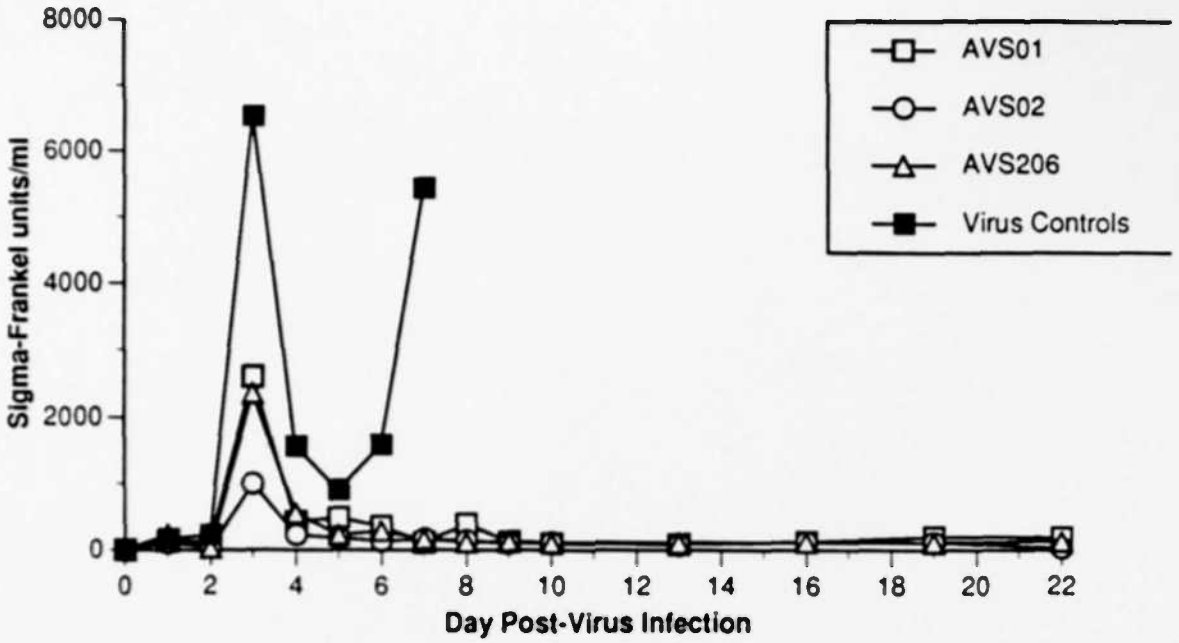


Figure VII-20. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on SGPT in Punta Toro Virus-Infected Mice.

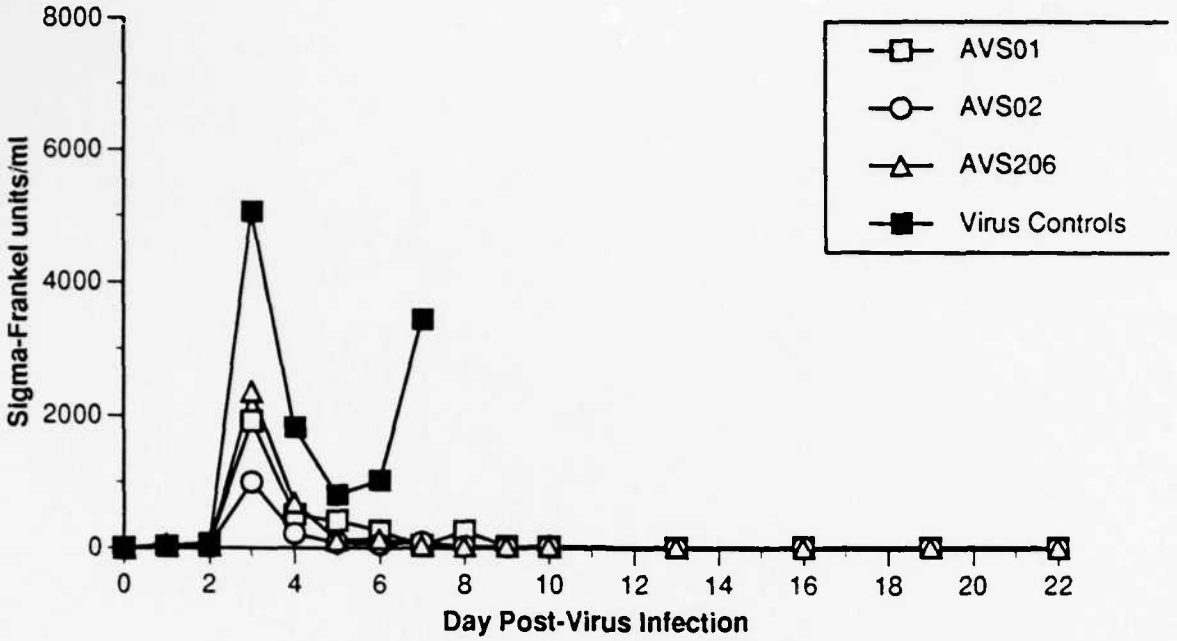


Figure VII-21. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on White Blood Cell Counts in Punta Toro Virus-Infected Mice.

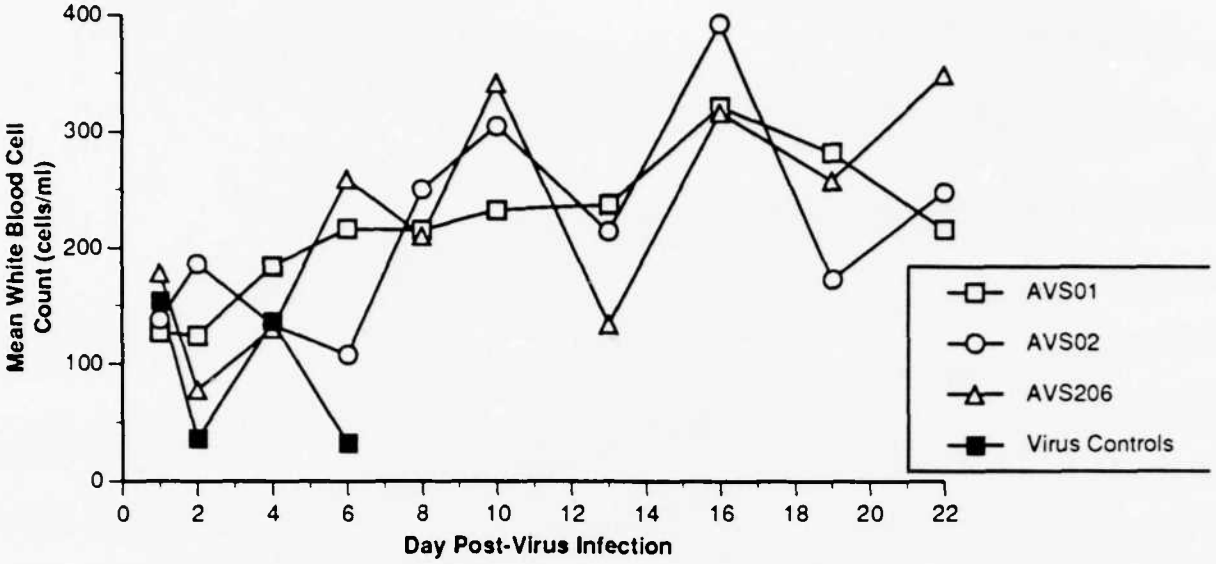


Figure VII-22. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Serum Virus Titers in Punta Toro Virus-Infected Mice.

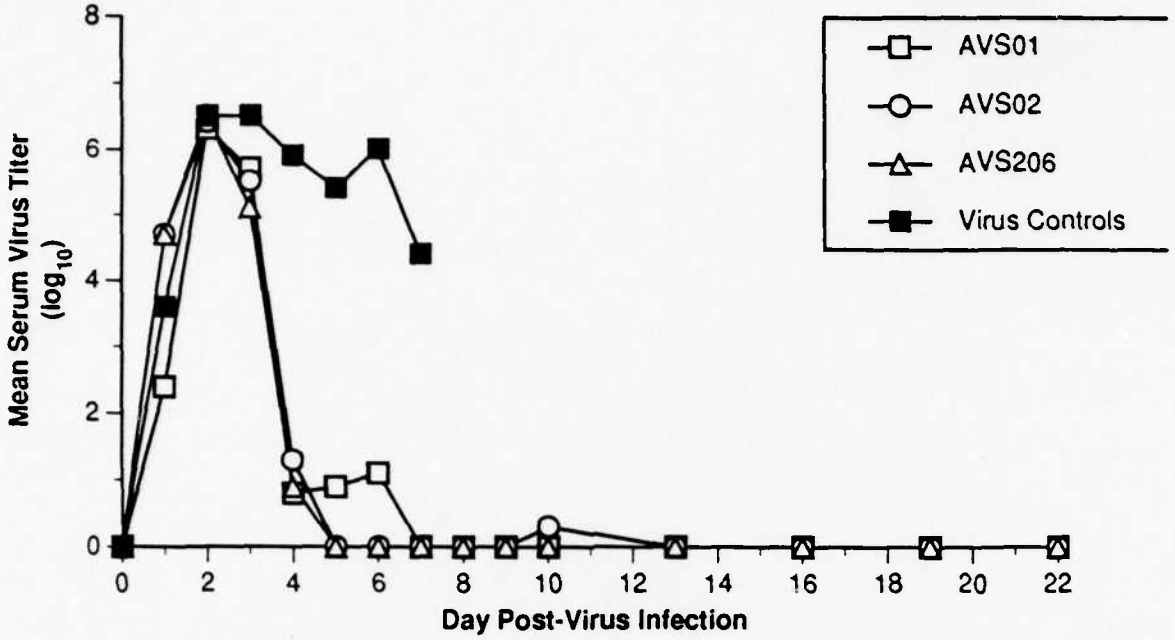


Figure VII-23. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Liver Virus Titers in Punta Toro Virus-Infected Mice.

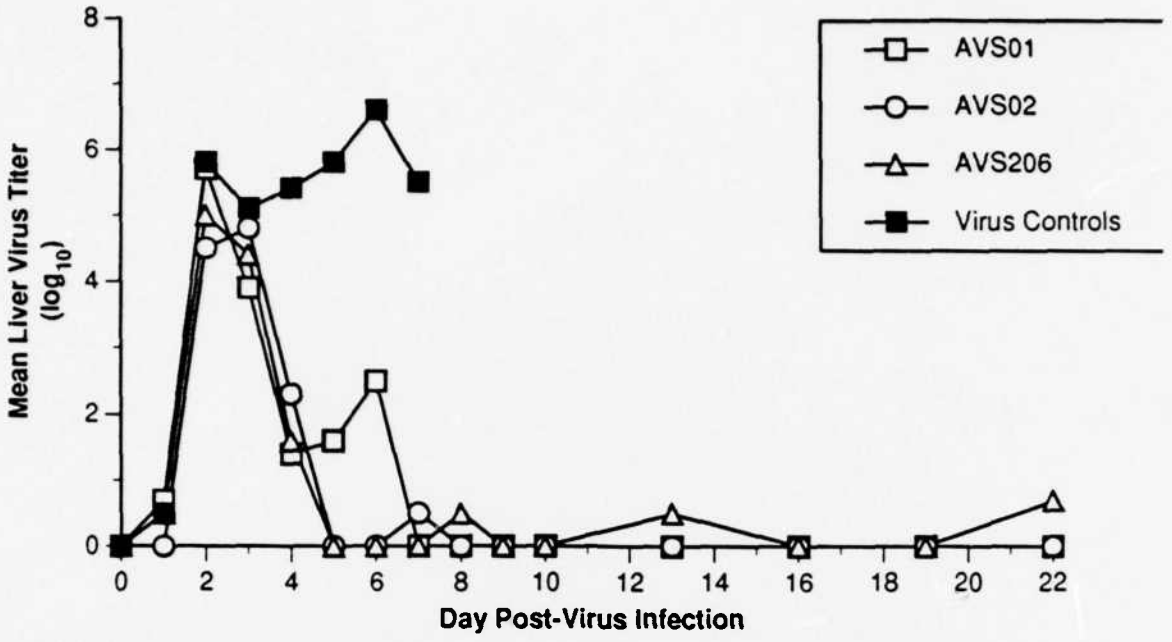


Figure VII-24. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Spleen Virus Titers in Punta Toro Virus-Infected Mice.

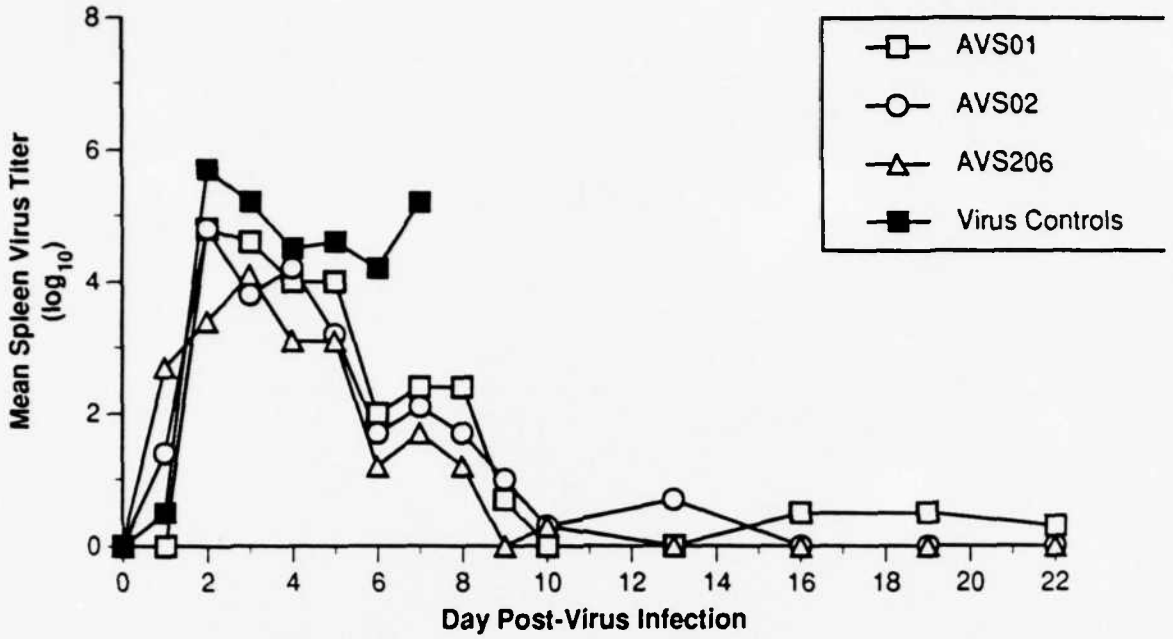


Figure VII-25. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Kidney Virus Titers in Punta Toro Virus-Infected Mice.

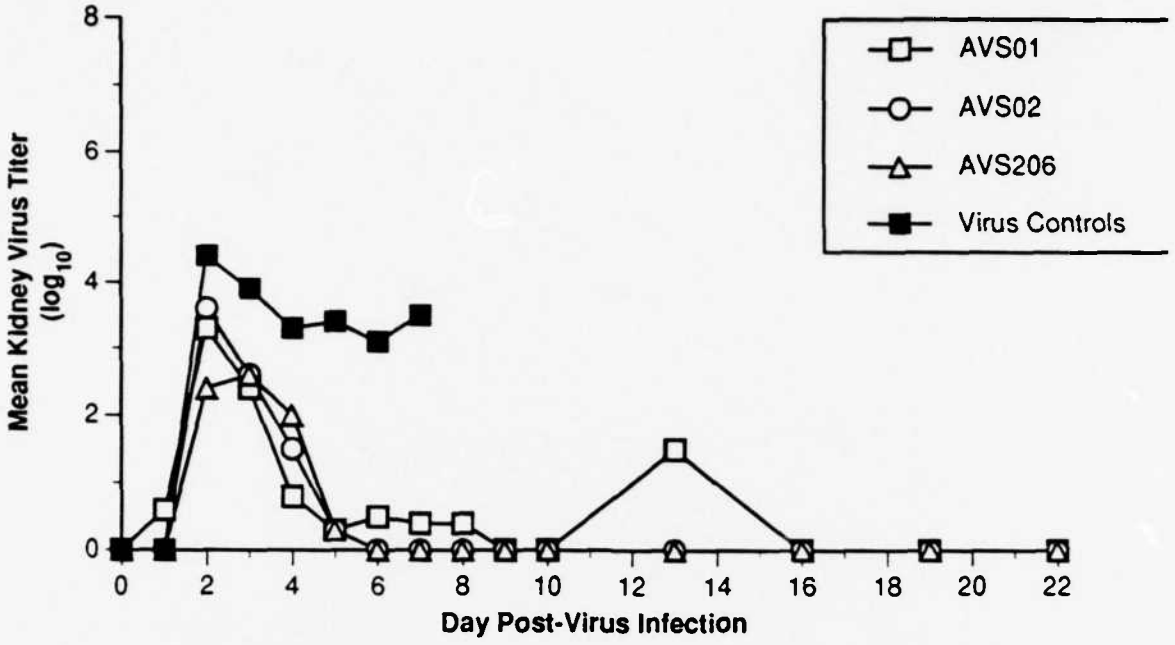


Figure VII-26. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Lung Virus Titers in Punta Toro Virus-Infected Mice.

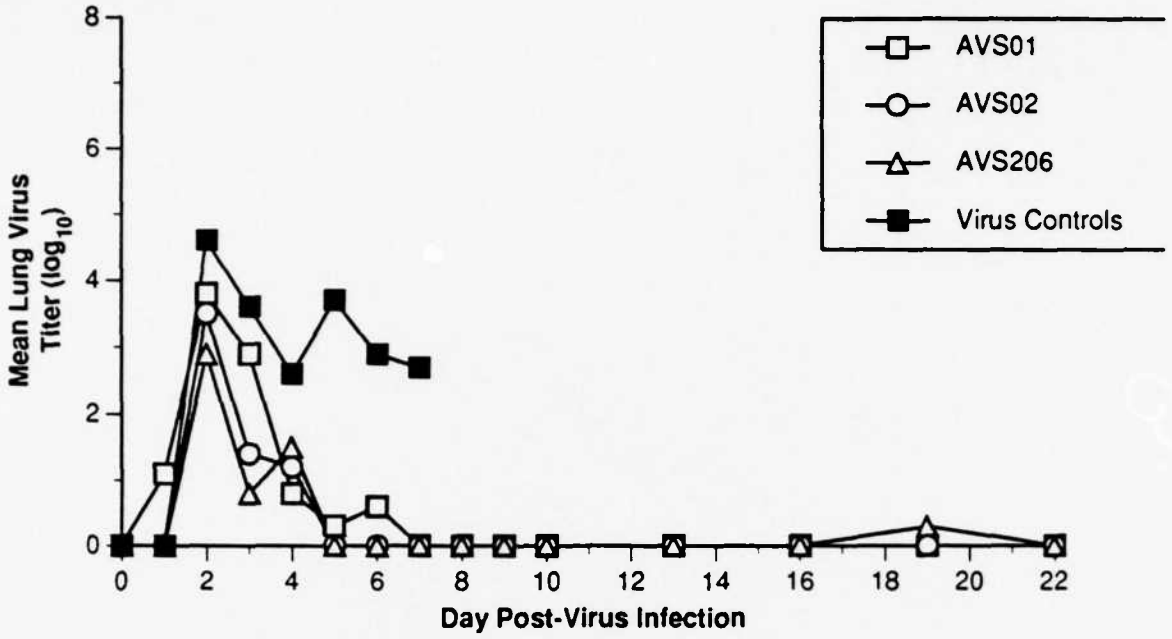


Figure VII-27. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Mesenteric Lymph Node Virus Titers in Punta Toro Virus-Infected Mice.

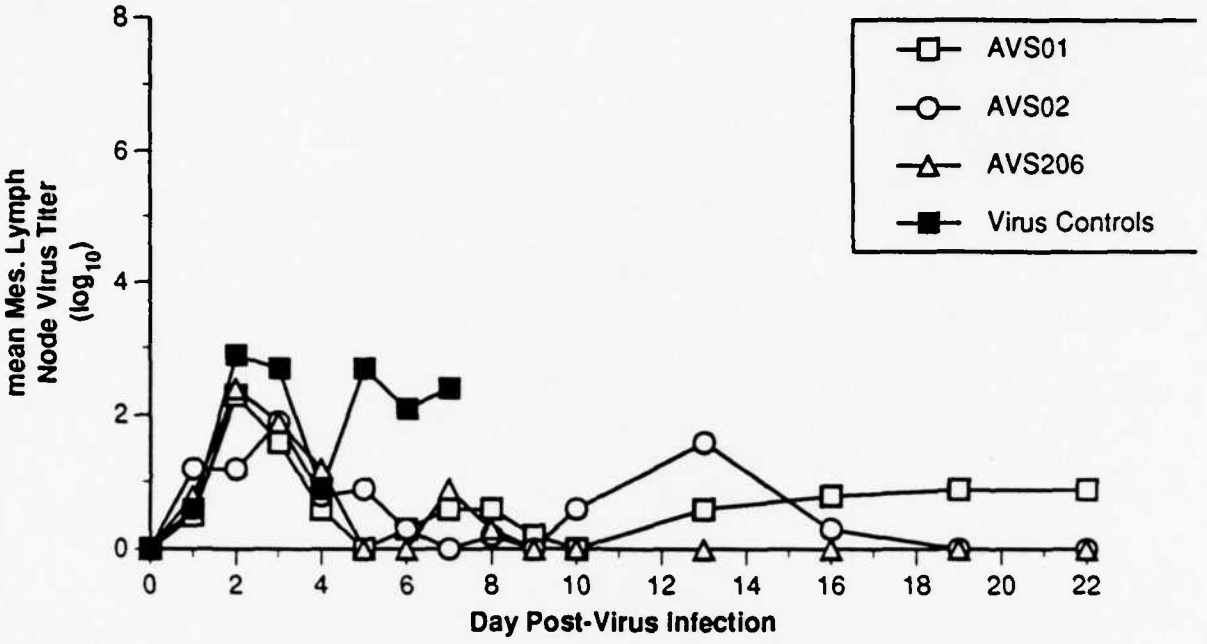


Figure VII-28. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Spinal Cord Virus Titers in Punta Toro Virus-Infected Mice.

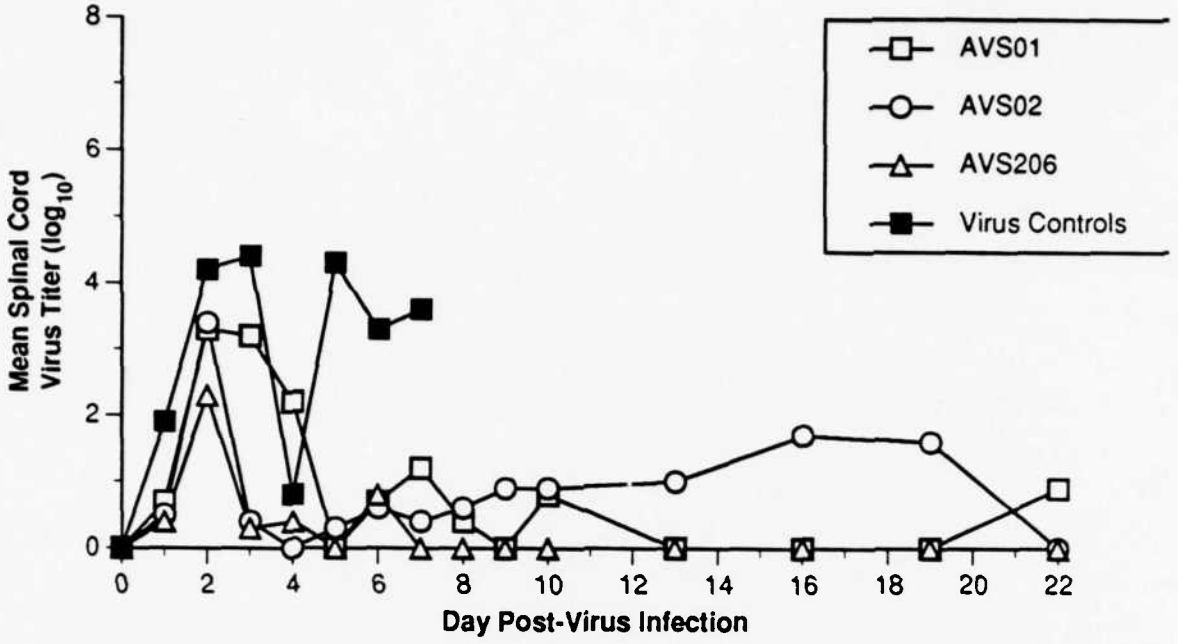


Figure VII-29. Expt. PtA771-773. Comparison of Effects of Single p.o. Treatments with AVS01, 02, and 206 on Brain Virus Titers in Punta Toro Virus-Infected Mice.

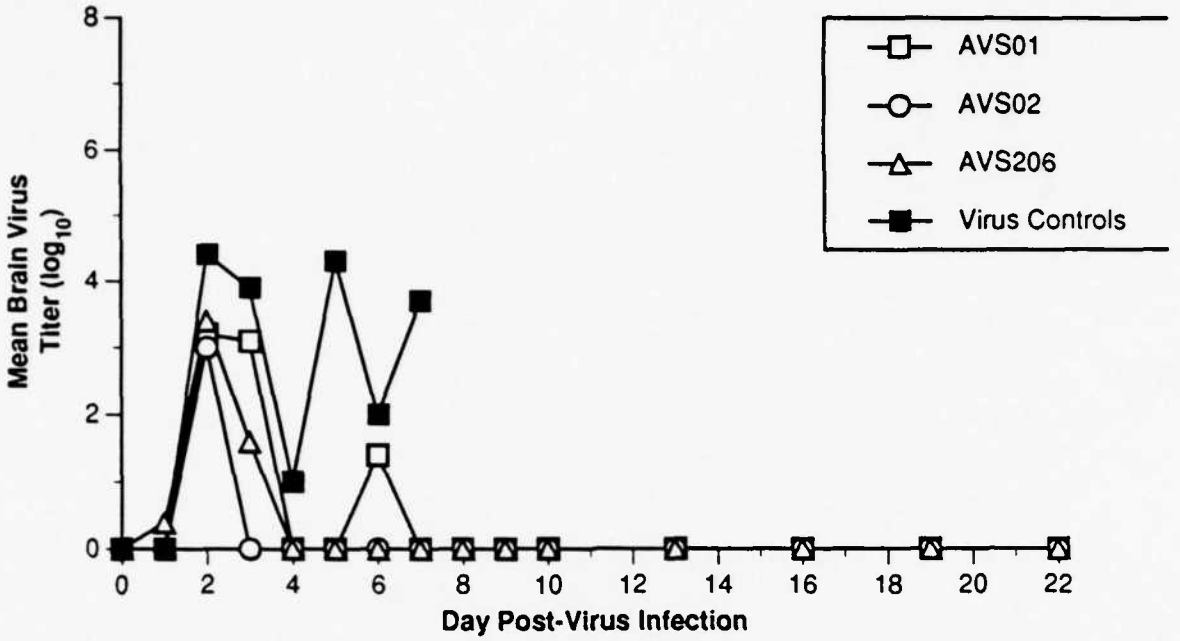


Table VII-4. Summary

1. Which has the greater THERAPEUTIC INDEX?

   s.c. bid x 5 death: AVS02
   day 2 virus titer reduction: AVS01 (2)[a], AVS02 (1)
   day 5 virus titer reduction: tie[c]
   day 2 SGOT, SGPT: AVS01
   day 5 SGOT, SGPT, liver score: tie

   p.o. bid x 5 death: AVS01, AVS206 (?)[b]
   day 2 virus titer reduction: tie
   day 5 virus titer reduction: tie (AVS206?)
   day 2 SGOT, SGPT: AVS206 (?)
   day 5 SGOT, SGPT, liver score: AVS01, AVS206 (?)

2. Which is most effective when TREATMENT IS DELAYED?

   s.c. qd x 5: AVS206
   p.o. qd x 5: tie (AVS01?)
   s.c. bid x 5: AVS02
   p.o. bid x 5: tie (AVS02?)

3. Which is most effective when TREATMENT DURATION IS REDUCED?

   p.o.: tie
   s.c.: tie

4. Which is most effective when VIRAL CHALLENGE IS INCREASED?

   AVS206 (?)

5. Which is most effective AGAINST I.C. INOCULATED BALLIET STRAIN PTV?

   AVS01

6. Which will better control the daily development of various PTV disease parameters?

   AVS206

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[a]() Indicates number of parameters where this compound was better than the others.

[b]? Indicates the differences were, at best, marginally better.

[c]tie = all three compounds were considered equal in their efficacy.

VIII. CHARACTERIZATION OF THE ADAMES STRAIN PUNTA TORO VIRUS INFECTION IN MICE

Introduction

It is important in running in vivo antiviral studies that a complete understanding has been achieved regarding the characteristics of the disease to be studied. This characterization should particularly be done in the animal species being used in the antiviral studies.

The PTV infection has been relatively well characterized by Pifat and Smith (1), the virus shown to induce a non-encephatic, lethal infection in mice that is characterized particularly by fulminant hepatocellar necrosis following peripheral inoculation into 3 week-old mice. These investigators showed that the C57BL/6 mice were perhaps most sensitive to the infection, although certain other mouse strains appeared also to be significantly affected by the infection.

This segment of our Final Report describes further studies we have accomplished to both characterize the disease further and to determine the rate at which various tissues in the mouse become infected.

Materials and Methods

Virus: The Adames strain of PTV as described in Section IV of this report was used.

Animals: C57BL/6, Swiss Webster, and NIH-III mice were used in various segments of this study. The former two strains were obtained from Simonsen Laboratories (Gilroy, CA); the NIH-III mice, a genetically immunodeficient mouse strain combining nude (*nu*) mutation making the athymic, the beige (*bg*) mutation reducing the number of NK cells, and the *xid* mutation reducing the number of LAK cells (2) were raised in our laboratory.

Enumeration of Phenotypic Frequencies (cell surface marker determinations): The frequencies of lymphocytes expressing the cell surface markers Thy-1 (total T-cells) and immunoglobulin (B-cells) were determined by flow cytometry enumeration: Splenic lymphocytes were adjusted to a concentration of 5×10^6 cells/ml in RPMI. A total of 100 µl of cell suspension were pipetted into each of five 12 x 75 mm test tubes. Splenic lymphocytes will be incubated with four µl of labeled anti-Thy-1 and anti-immunoglobulin. A non-specific rat monoclonal of the same subclass and unlabeled cells served as controls. The percent positive labeled cells were determined with an EPICS-C flow cytometer (Coulter Electronics Inc., Hialeah, FL), equipped with an argon laser tuned to 400 mW at the 488 nm line.

B and T Cell Response Determinations: Several mitogens and antigens selectively stimulate different lymphocyte subpopulations to proliferate. Some mitogens stimulate T-cells (concanavalin A (Con A)), and others such as LPS are specific for B lymphocytes. Thus, it is possible to assess B-cell function independently of T-cell function. The blastogenic response of spleen cells to various concentrations of *E. coli*, LPS, and Con A were determined to assess general B and T cell function. A total of 5×10^5 pooled spleen cells in 0.1 ml volumes of RPMI-1640 medium with 10% FBS and 2-mercaptoethanol were added to triplicate wells of 96-well flat-bottomed microplates. LPS or Con A diluted to 2-25 µg/ml in 0.1 ml volumes of 10% FBS in RPMI-1640 were added to wells. Also included were wells containing only medium to serve as controls. The plates were incubated for 48 hr at 37°C, then pulsed with [^{3}H]thymidine, harvested and counted.

Determination of IL-2 Production: Splenic lymphocytes from infected animals were tested for their ability to produce IL-2 by incubating them (2×10^6 cells) in 2 ml of RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% phytohemagglutinin (PHA), and 2-mercaptoethanol. After 48 hr at 37°C, the supernate was harvested, centrifuged at 500 x g for 5 minutes to remove cells, and assayed for IL-2. The IL-2 assay was done by adding 0.1 ml of serial 2-fold dilutions of the supernate to triplicate wells in 96-well flat-bottomed microplates, after which 4×10^4 HT-2 cells in 0.1 ml medium were added to each well. The HT-2 cells, a murine Balb/c cloned cell line, is IL-2 dependent for its growth. The cell-supernate mixture was incubated at 37°C for 20 hr, pulsed with [^{3}H]thymidine, incubated 4 more hr, and the radiolabel uptake determined.

Antibody Assay: Each dilution of heat inactivated (56°C for 30 min) serum was mixed with an equal volume of Adames strain PTV. The virus concentration was 100 cell culture 50% infectious

doses (CCID50)/0.1 ml. The mixture was incubated for 1 hr at 37°C, the 0.2 ml was added to each of 4 cups in a 96-well microplate containing a monolayer of LLC-MK_2 cells in test medium. The cells were incubated 7 days and viral CPE read. The 50% endpoint was determined using the Reed Muench method.

IFN Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only.

Virus Assay: Tissues were homogenized to a 10% (wt./vol.) suspension prepared in minimum essential medium (MEM); the samples were assayed for PTV by diluting each 10-fold to a titer of 10^{-5}; 0.2 ml of each dilution was added to triplicate cups of LLC-MK_2 cell monolayers in 96-well microplates. Viral cytopathic effect was determined after 5 days' incubation at 37°C and 50% endpoints were then determined.

Hepatic Icterus Scoring: Livers removed from infected mice were examined for the degree of discoloration seen, this scored from 0 (normal) to 4 (maximal discoloration).

Determinations of SGOT, SGPT: Titration of these transaminase enzymes was accomplished using colorimetric kits from Sigma Chemical Co. (St. Louis, MO). Spectrophotometric readings for these colorimetric assays were performed in duplicate by using a microplate autoreader (EL309, Bio-Tek Instruments, Inc., Winooski, VT).

Experiment Design: The various disease characteristics to be described in this section are the result of numerous experiments, the protocol for each described in some detail on the tables and figures in this section.

Results and Discussion

Development of death in C57BL/6 mice: (Figure VIII-1). Mice inoculated s.c. with a lethal dose of PTV began dying 3 days after virus inoculation, and continue dying through day 7. Mean day of death was approximately day 4.

Development of hepatic icterus and increased SGOT and SGPT: (Figure VIII-2). Liver discoloration began to be evident in the PTV-infected mice by 2 days after virus inoculation, reaching a peak having a mean score of near 4+ by 5 days; this was accompanied by marked increases in levels of SGOT and SGPT.

Virus development in serum and tissues: Virus in the serum of PTV-infected mice achieved titers in excess of 10^6 cell culture infectious doses (CCID50) by 2 days after virus inoculation (Figure VIII-3A); due to this high titer, before tissues were taken from infected mice for viral assay, the animals were anesthetized using a mixture of ketamine (20 mg/ml), acepromazine (0.3 mg/ml), and rompom (1 mg/ml) in water injected i.p. in a volume of 0.05 ml/mouse. When the animals showed no reflexes, they were pinned and their chest cavities opened. The right atrium was cut and simultaneously saline was injected into the left ventricle. The animals were considered perfused when saline appeared in the right atrium (approximately 10 minutes). This was done to attempt to remove as much blood from the tissues as possible to avoid contaminating the tissue with excess virus. Virus was subsequently found in the following tissues: liver (Figure VIII-3A), spleen (Figure VIII-3B), kidney (Figure VIII-3C), lung (Figure VIII-3D), mesenteric lymph node (Figure VIII-3E), spinal cord (Figure VIII-3F), and brain (Figure VIII-3G). White blood cells washed 3 times were also found to be infected on day 2 of the infection, the mean virus titer being approximately 10^2 CCID50/10^6 cells (Figure VIII-3A).

Development of serum neutralizing antibody: Antibody was present by the first week after infection of 4 week-old C57BL/6 mice and persisted at a titer of >2 $\log_{10}$ units/50 µl of serum through 28 days (Table VIII-1). This was considered a relatively rapid antibody titer development

and suggests such antibody may play a role in recovery of the animals from the infection. It should be pointed out that this study was run in 4 week-old mice which are somewhat more resistant to PTV infection than are 3 week-old mice used in our standard antiviral experiments. The older mice were chosen to assure us that some would be alive for the later antibody assays. It is important to also determine this antibody titer development in the younger mice.

Hematologic changes in C57BL/6 mice: (Figure VIII-4). As Pifat and Smith (2) have reported, infection with PTV results in a profound suppression of total white blood cells (WBC) and lymphocytes. Our data also indicate T cells and suppressor/cytotoxic T cells also are suppressed at the same time. It is noted on day 1 of the infection, the WBC and lymphocytes increased quite dramatically, probably in response to the infection. The marked suppression occurring by day 2 suggests the virus may actually attack these cells, resulting in their destruction. As described above, WBC indeed contain detectable PTV on day 2 of the infection.

Effect on other immunologic parameters: A large pool of mice infected with PTV was used to provide 10 mice killed once daily for 5 days. The spleens were removed, suspended in RPMI-1640 medium, and dissociated by use of a tissue homogenizer. The spleen cell preparations were pipetted onto nylon wool columns and incubated 1 hr at 37°C. Non-adherent cells were eluted from the columns with warm RPMI-1640 medium and further treated by hypotonic lysis to eliminate red blood cells, then assayed for T and B cell enumeration, B cell response to lypopolysaccharide, T cell response to concanavalin A, and IL-2 production. The mean cell enumeration results are seen in Figure VIII-5. The percentage of splenic T cells declined by nearly 20% following the infection beginning after the 2nd day. The percentage of B cells also declined, but to a lesser extent (10%) during the same period of time.

The B- and T-cell functions declined precipitously by the first day of infection, as seen in Figure VIII-6. IL-2 production also dropped, but not as rapidly as the other functions (Figure VIII-6). Since IL-2 acts to amplify the response of B and T cells to mitogens, the latter decline may be a result of decreasing IL-2 production. Although not shown on the figures, all function tests were run on the same numbers of spleen cells, so these declines are not a manifestation of the less cell numbers seen in Figure VIII-5.

As pointed out earlier in this section, spleens do become infected with PTV, with relatively high titers of infectious virus recovered from the spleen. The data here suggest that the PTV infection may be affecting cell function as well as cell number. Importantly, the animals are apparently significantly immunosuppressed by this *Phlebovirus* infection. These data imply that treatment with immunomodulators that would reverse these immunosuppressive effects may be good candidates for anti-PTV therapy.

Serum interferon production: A large pool of male and female 3 and 4 week-old C57BL/6 mice were infected s.c. with PTV, then groups of 10 were killed 2, 4, 8, 24, 36, and 48 hr later. Their liver scores were determined and the livers assayed for virus titer. The serum was assayed individually for IFN titer.

The results are summarized in Tables VIII-2 and VIII-3. Livers scores had just begun to develop by 36 hr after virus inoculation, with the smaller mice displaying higher scores as might be anticipated from our results reported in another section of the increased sensitivity of mice with lower weights to PTV. Liver virus titers also developed at about this same time, with high titers recovered by 36 hr and 48 hr.

Interferon was seen in all groups at somewhat similar titers by 48 hr. These data indicate that weight differences apparently did not influence the animals' ability to induce IFN. We have not attempted to differentiate the types of IFN induced by these animals. It is possible there may be differences in production of that IFN type which is more protective to the animals. Pifat and Smith (1) used combined anti-IFN-α and anti-IFN-β to ablate the resistance to PTV in 8 week old mice, so we are unsure which IFN was the more protective. These investigators found both 4- and 8-week-old mice produced IFN at about the same rate, with the 4-week-old mice succumbing to the infection despite the high IFN titers. Certainly in our studies with mice in the weight ranges used in this study the mice die within 5-8 days of the infection despite the IFN produced. Thus, IFN alone may not be the only means by which these animals survive PTV infection.

Effects of animal weight on PTV lethality: Mice were separated into groups weighing 4 to 8 grams and 12 to 15 grams. Our standard pool of PTV was then titrated in each group with 10 mice

infected i.p. with 0.1 ml of each one-half $\log_{10}$ dilution of virus. Deaths were noted daily through day 21.

The results of this dual titration are summarized in Table VIII-4. The smaller mice were markedly more sensitive to the virus than the larger animals. In the latter animals, the deaths which occurred were highly erratic. A 2 $\log_{10}$ difference was seen between the titer of the virus in the two groups.

These data indicate that animal size, which may or may not reflect the age of the animal, is a major factor in influencing the degree of sensitivity of C57BL/6 mice to PTV infections.

This experiment was repeated in Swiss Webster mice weighing 9-11 g or 14-17 g. The results of this titration are seen in Table VIII-5. Again, the younger mice were more susceptible to the infection, but in neither group did sufficient animals die to warrant changing to this strain of mice for our antiviral studies.

Sensitivity of NIH-III mice to PTV infection: Four or 5 mice were injected with each of 5 dilutions of PTV, then held and observed for death over a 21-day period under sterile holding conditions.

The results of this titration are summarized in Table VIII-6. The high concentrations of PTV did not cause death in the mice. However, the 10^{-3} concentration apparently killed one animal, and the 10^{-4} concentration killed all mice injected. We have encountered a "window" of sensitivity in the past using this virus in other mouse strains. In such situations, the higher concentrations (usually 10^{0} or 10^{-1}) were less lethal to the mice than were lower concentrations, suggesting the presence of defective interfering particles. These results with NIH-III mice, however, indicate a much exaggerated "window", if indeed the deaths were due to PTV. This could be due to some immunologic defect in the mice. We will repeat this experiment using lower virus concentrations.

Ease of PTV transmission among mice: Normal mice were caged 5 to a cage and their cages placed next to cages of PTV-infected mice. These "sentry" mice were exposed to PTV-infected mice for periods of time ranging from 6 weeks to one year. At the dates indicated, the sentry mice were killed and their serum assayed for anti-PTV antibody. As a control, human serum containing anti-PTV antibody provided by Dr. Pifat of USAMRIID was run in parallel. The sera and livers of several sentry mice were also assayed for infectious PTV.

The results of this study are summarized in Table VIII-7. The known positive serum sample had an antibody titer of >2.2 $\log_{10}$ units/0.5 ml, but no sentry mice tested of 23 had demonstrable antibody titers. Six livers and serum samples assayed for virus were found to be negative.

These results suggest PTV is not readily transmitted, at least cage to cage. This is in contrast to murine hepatitis virus and epizootic diarrhea virus of infant mice, which spreads readily from cage to cage.

Conclusions

The disease induced by s.c. or i.p. inoculation of the Adames strain of PTV into 3 week-old C57BL/6 mice is acute, characterized by rapid rise of virus in all tissues and in blood, major liver failure within 3-5 days of infection, and subsequent death of the animal by 3 to 6 days. The infection is profoundly immunosuppressive, as seen by decreasing total splenic T and B cells, reduced B and T cell function, declining ability of splenic cells to produce IL-2. The cell decreases were seen by day 3 of the infection, the functionality declines by day 1. Older mice, Swiss Webster and NIH-III mice do not appear to be as sensitive to the disease as the C57BL/6 species. The infection is apparently not readily transmitted between cages.

Literature Cited

1. Kamel-Reid, S. and J.E. Dick. 1988. Engraftment of immune-deficient mice with human hematopoietic stein cells. Science 242:1706-1707.
2. Pifat, D.Y. and J.F. Smith. 1987. Punta Toro virus infections of C57BL/6 mice: A model for phlebovirus-induced disease. Microb. Pathogen. 3:409-422.

Figure VIII-1. Occurrence of Death in 3 Week-Old C57BL/6 Mice Infected with PTV

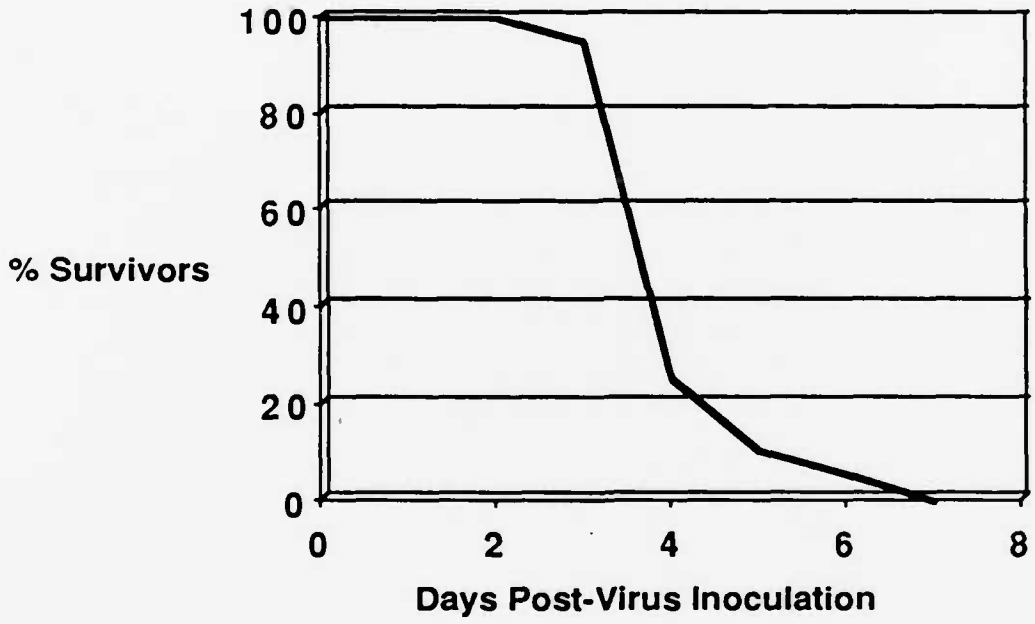


Figure VIII-2. Development of Serum Glutamic and Pyruvic Transaminases (SGOT, SGPT) and Hepatic Icterus in 3-Week-Old C57BL/6 Mice Infected with PTV

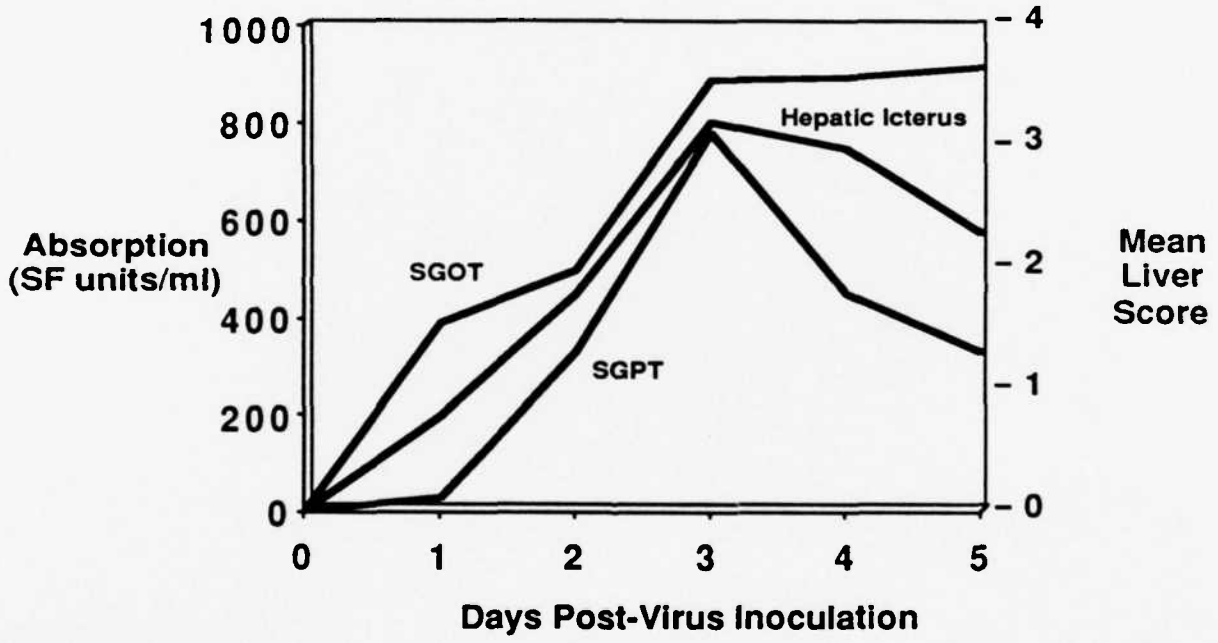


Figure VIII-3A. Recoverable Virus From Sera, Livers, and Peripheral White Blood Cells of 3 Week-Old C57BL/6 Mice Infected with PTV

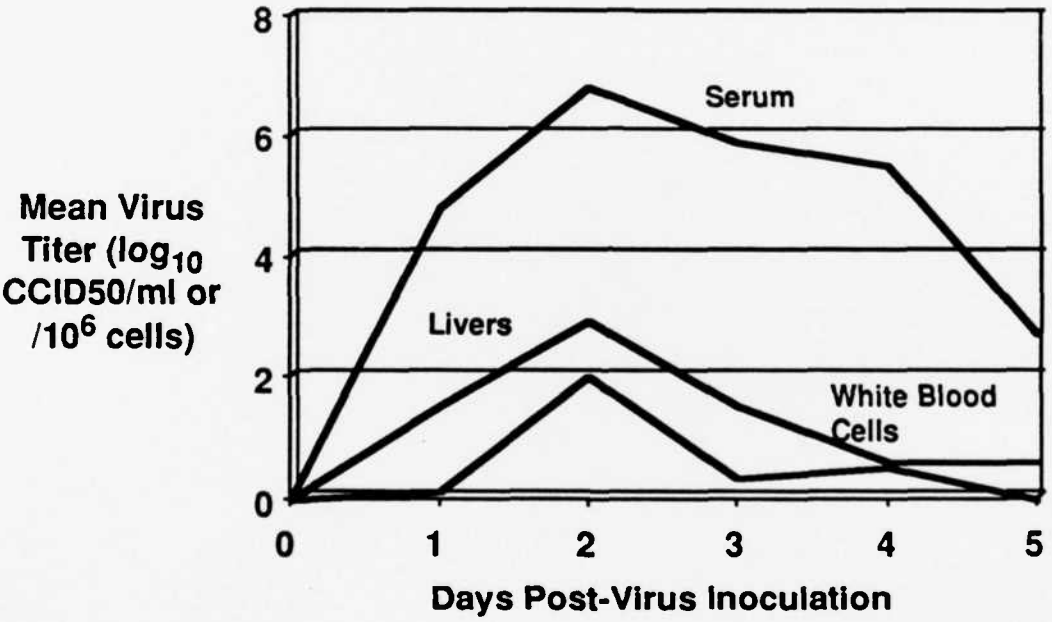
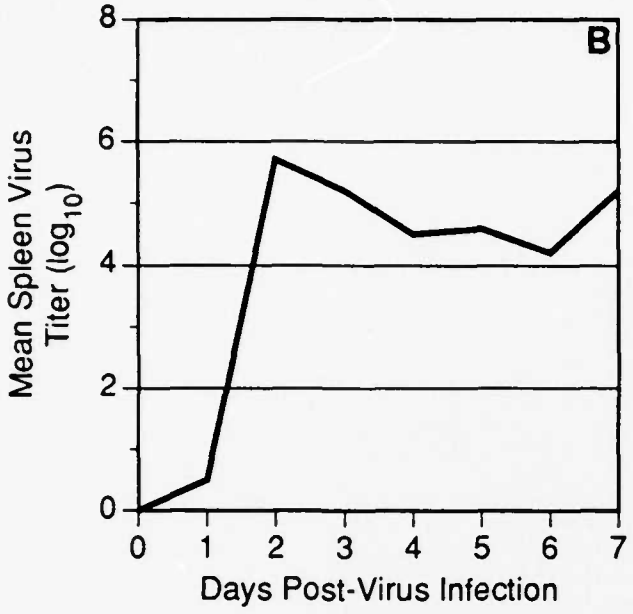
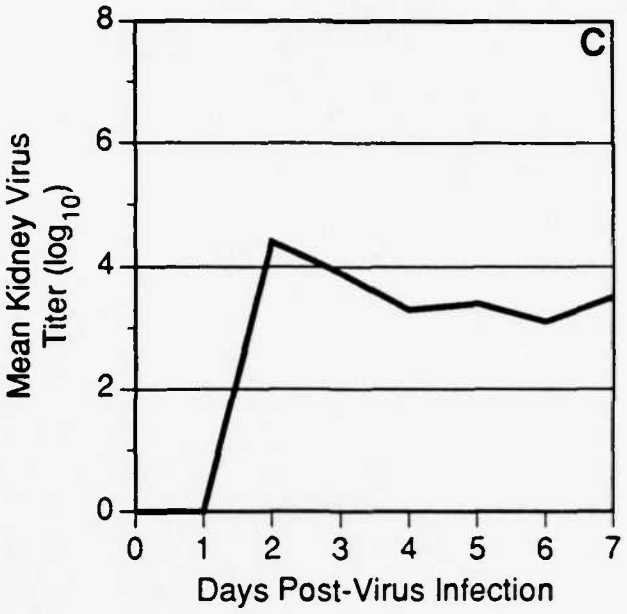


Figure VIII-3B-D. Virus Titers in Punta Toro Virus-Infected C57BL/6 Mice. (B: Spleens; C: Kidney; D: Lungs)





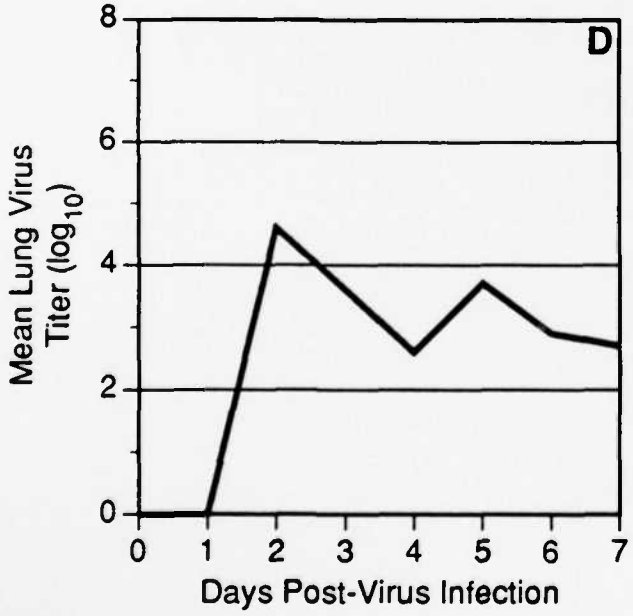
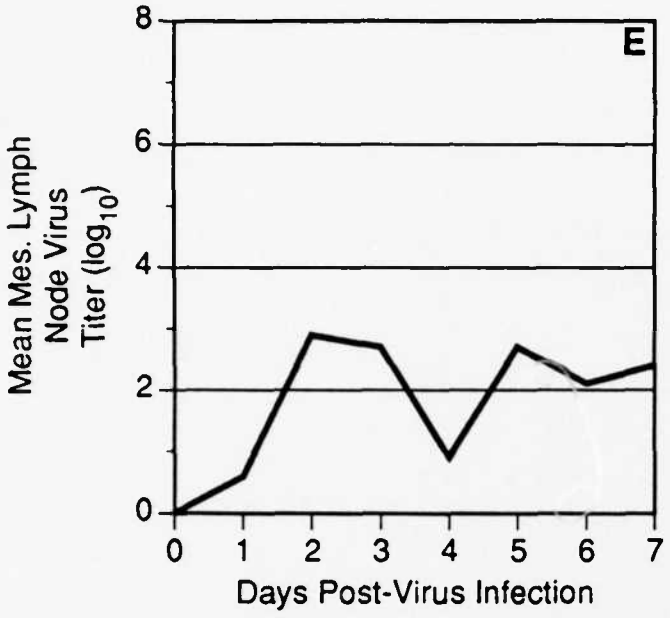
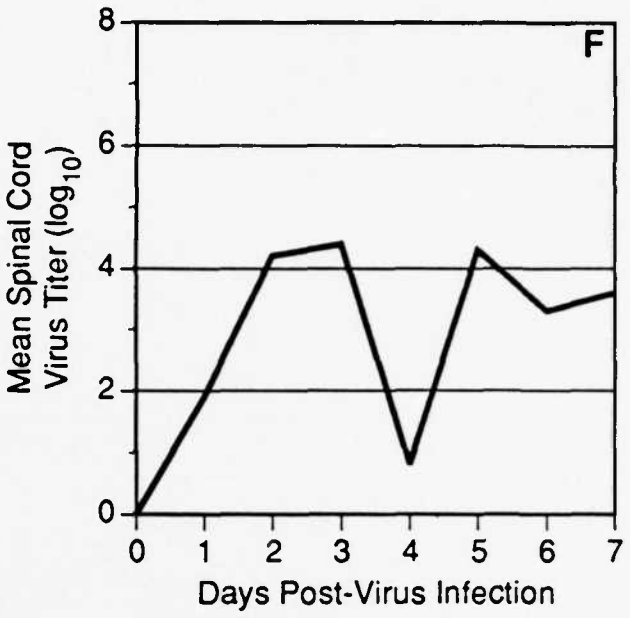


Figure VIII-3E-G. Virus Titers in Punta Toro Virus-Infected C57BL/6 Mice. (E: Mes. Lymph Node; F: Spinal Cord; G: Brains)





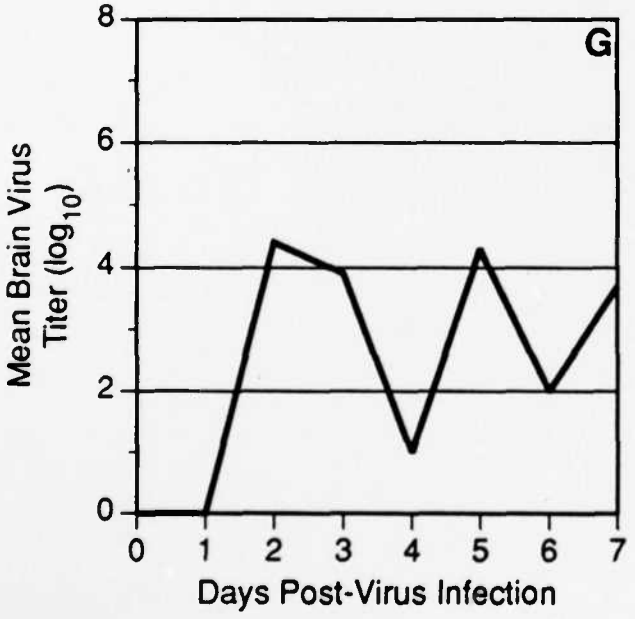


Table VIII-1. Expt. PT153. Neutraiizing Antibody Production in Punta Toro Virus-Infected C57BL/6 Male Mice.

Animals: 4 Week-old male C57BL/6 mice.

Virus: PTVA/4LLC 8-19-87, s.c. inoculated. Dilution $10^{-3.0}$.

Time Post-Virus Inoculation (days)	Neutralizing Antibody (log_{10} units/50 µl)
7	1.2
14	1.6
21	2.0
28	2.1

Figure VIII-4. PTV-Induced Hematologic Changes in 3-Week-Old C57BL/6 Mice

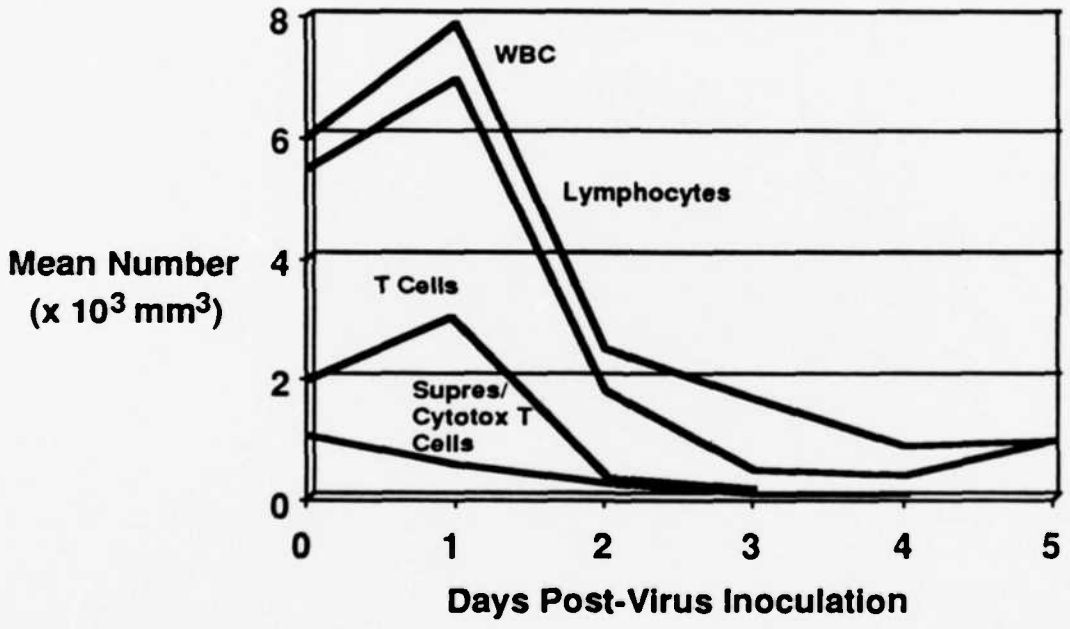


Figure VIII-5. PTV-Induced Changes In Percentage of Spleen T and B Cells in 3 Week-Old C57BL/6 Mice.

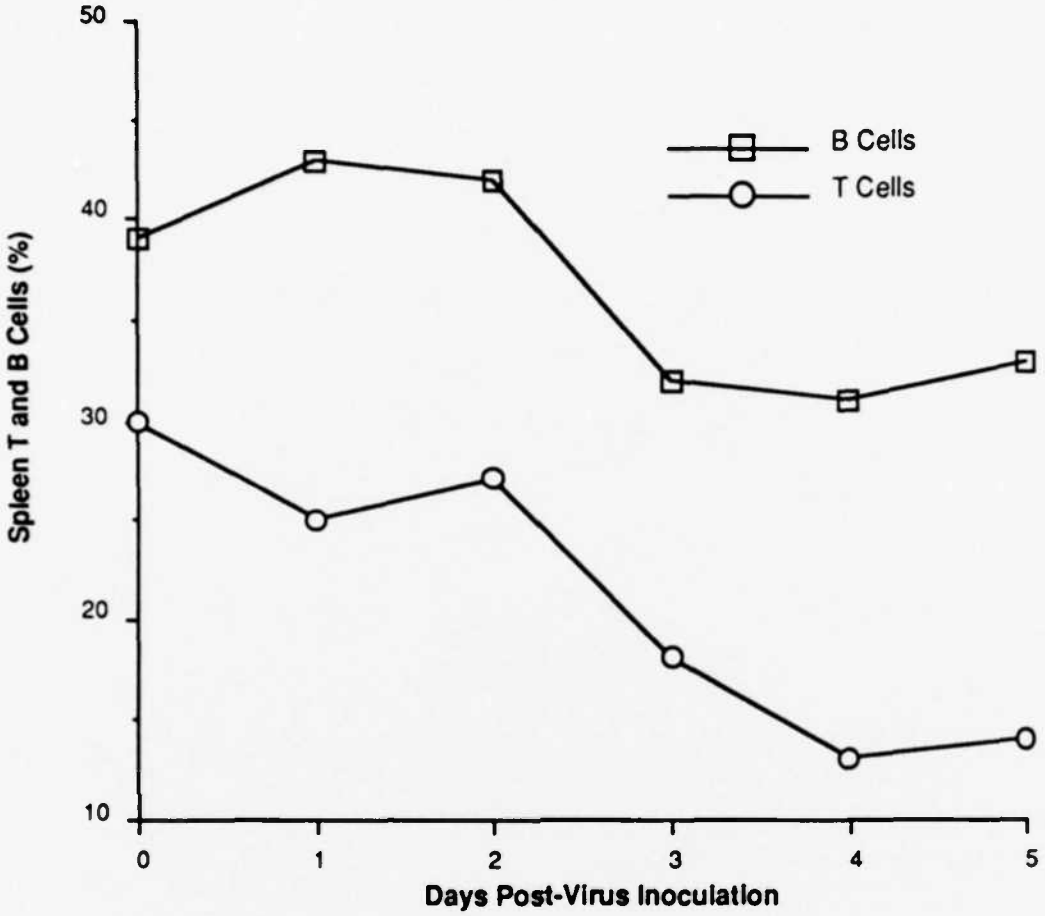


Figure VIII-6. PTV-Induced Changes in LPS and Con A Response and on Interleukin-2 Production in 3 Week-Old C57BL/6 Mice.

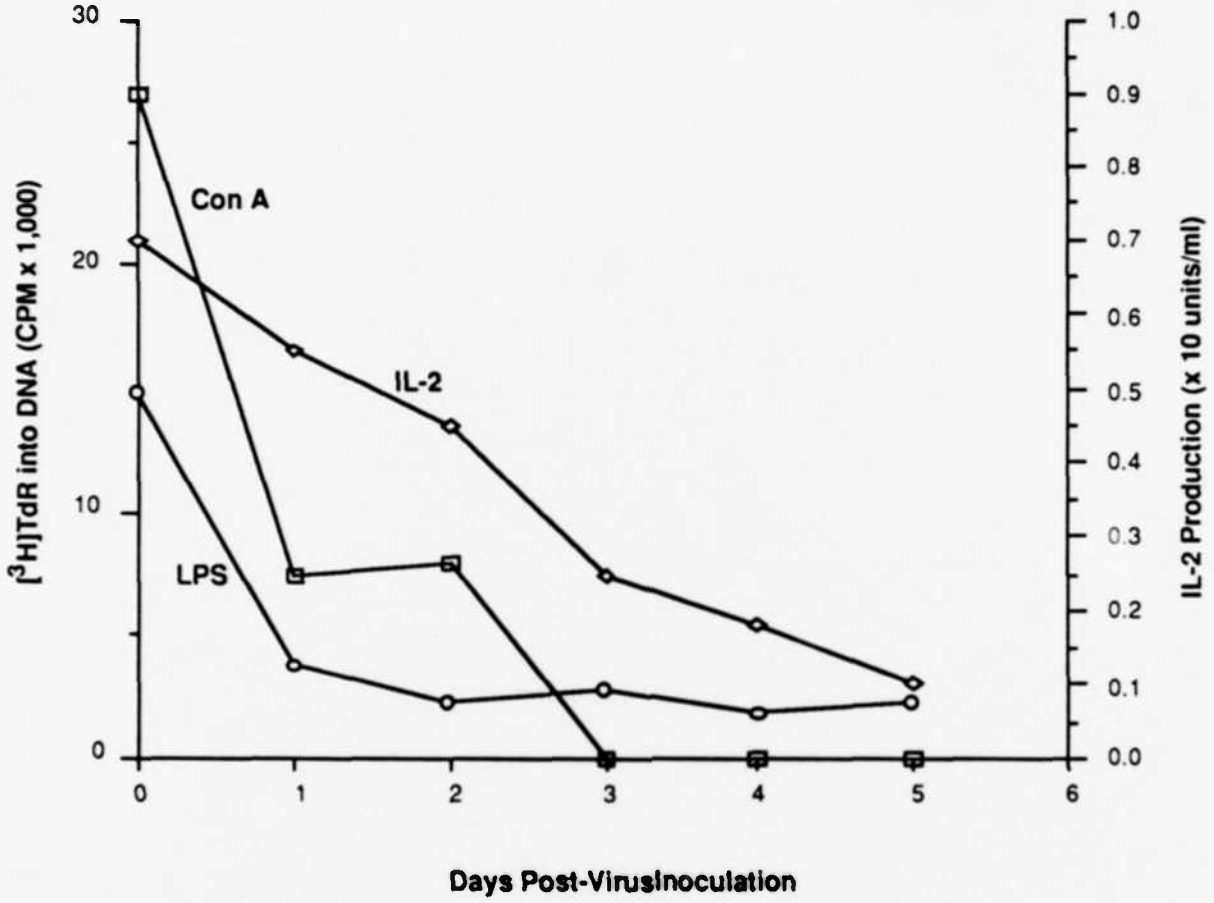


Table VIII-2. Expt. PT145-146. Serum Interferon Production, Liver Score, and Liver Virus Titer in 9-11 g Male or Female Punta Toro Virus-Infected C57BL/6 Mice.

Animals: 3 Week-old 9-11 g C57BL/6 mice. Virus: PTVA/4LLC 8-19-87, s.c. inoculated. Dilution $10^{-3.0}$.

Time Post-Virus Inoculation (hrs)	IFN Titer[a] (log_{10} units/0.1 ml)	Mean Liver Score[b]	Mean Liver Virus Titer[c]
Female Mice			
2	0.0	0.0	0.0
4	0.0	0.0	0.0
8	0.0	0.5	0.0
24	0.0	0.0	0.8
36	0.0	0.8	3.6
48	3.7	0.7	5.8
Male Mice			
2	0.0	0.0	0.4
4	0.0	0.0	0.0
8	0.0	0.1	0.3
24	0.0	0.0	0.5
36	1.6	0.4	5.1
48	4.5	0.5	6.2

[a]IFN assayed by testing different serum dilutions in mouse L cells using vesicular stomatitis virus inhibition as endpoint.

[b]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed.

[c]Log_{10} geometric mean.

Table VIII-3. Expt. PT147, 149. Serum Interferon Production, Liver Score, and Liver Virus Titer In 14-16 g Male or Female Punta Toro Virus-Infected C57BL/6 Mice.

Animals: 4 Week-old 14-16 g C57BL/6 mice. Virus: PTVA/4LLC 8-19-87, s.c. inoculated. Dilution $10^{-3.0}$.

Time Post-Virus Inoculation (hrs)	IFN Titer[a] (log_{10} units/0.1 ml)	Mean Liver Score[b]	Mean Liver Virus Titer[c]
Female Mice			
2	0.0	0.0	0.0
4	0.0	0.4	0.0
8	0.0	0.2	0.0
24	0.0	0.1	0.7
36	0.0	0.2	2.8
48	4.8	0.2	6.1
Male Mice			
2	0.0	0.1	0.0
4	0.0	0.2	0.0
8	0.0	0.1	0.0
24	0.0	0.4	0.0
36	0.0	0.3	1.3
48	3.1	0.6	5.9

[a]IFN assayed by testing different serum dilutions in mouse L cells using vesicular stomatitis virus inhibition as endpoint.

[b]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed.

[c]Log_{10} geometric mean.

Table VIII-4. Effect of Animal Weight on Lethality of Adames PTV in C57BL/6 Mice.

Weight Range: 4-8 g

Virus Dilution	Dead/ Total	Mean Survival Time
$10^{-1.0}$	5/10	6.6
$10^{-1.5}$	5/10	5.8
$10^{-2.0}$	9/10	5.1
$10^{-2.5}$	10/10	4.5
$10^{-3.0}$	10/10	4.0
$10^{-3.5}$	10/10	4.1
$10^{-4.0}$	10/10	4.2
$10^{-4.5}$	10/10	4.0
$10^{-5.0}$	10/10	4.8
$10^{-5.5}$	9/10	5.0
$10^{-6.0}$	9/10	5.3

LD50 = $>10^{-6.0}$

Weight Range: 12-15 g

Virus Dilution	Dead/ Total	Mean Survival Time
$10^{-1.0}$	6/10	6.2
$10^{-1.5}$	8/10	5.6
$10^{-2.0}$	9/10	4.6
$10^{-2.5}$	5/10	5.6
$10^{-3.0}$	7/10	6.0
$10^{-3.5}$	8/10	3.4
$10^{-4.0}$	6/10	6.7
$10^{-4.5}$	9/10	5.4
$10^{-5.0}$	2/10	7.5
$10^{-5.5}$	4/10	6.5
$10^{-6.0}$	2/10	6.0

LD50 = $10^{-4.2}$

Table VIII-5. Infectivity of PTV in Swiss Webster Mice.

Three week-old mice

Virus Dilution	Survivors/Total	Mean Surv. Time (days)
10^{-2}	7/10	5.3
10^{-3}	9/10	6.0
10^{-4}	8/10	5.0
10^{-5}	4/10	4.5
10^{-6}	4/10	4.3

Four week-old mice

Virus Dilution	Survivors/Total	Mean Surv. Time (days)
10^{-2}	10/10	>21.0
10^{-3}	9/10	6.0
10^{-4}	9/10	5.0
10^{-5}	9/10	4.0
10^{-6}	9/10	5.0

Table VIII-6. Susceptibility of NIH-III Mice[a] to s.c. PTV Inoculation.

Virus Dilution	Surv/ Total	Mean Surv. Time
10^0	4/4	>21
10^{-1}	4/4	>21
10^{-2}	4/4	>21
10^{-3}	3/4	8.0
10^{-4}	0/5	4.4

[a]5-8 month-old females.

Table VIII-1. Punta Toro Sentry Mouse Titration Data

Expt. No.	Description	Date In	Date Sacrificed	Virus Titer (log_{10} $CCID_{50}$/0.1ml) Serum	Virus Titer (log_{10} $CCID_{50}$/0.1ml) Liver	Neutralizing Antibody Titer (log_{10} Units/0.5ml)
PT #1	Cage 1, ♂	5/24/86	7/15/86		<1.7, <1.7, <1.7	
		"	5/11/87	<0.7[a]		<1.0, <1.0, <1.0, <1.0 <1.0, <1.0, <1.0, <1.0
	Cage 2, ♀	5/24/86	7/15/86	<0.7[b]	<1.7, <1.7, <1.7	
		"	5/11/89			<1.0, <1.0, <1.0
	Cage 3, ♂	5/24/86	5/11/87	<0.7[c]		<1.0, <1.0
PT #2	Cage 1, ♂	6/4/86	5/11/87	<0.7		<1.0, <1.0, <1.0
	Cage 2, ♀	6/4/86	5/11/87	<0.7[b]		<1.0, <1.0, <1.0
PT #3	Cage 1, ♀	7/2/86	5/11/87	<0.7[c]		<1.0, <1.0
Normal ♀ mouse serum						<1.0
D.P. Pos. Serum of 12/81 (human anti-Punta Toro virus serum)						>2.2

[a]Serum samples from 8 mice were pooled.

[b]Serum samples from 3 mice were pooled.

[c]Serum samples from 2 mice were pooled.

IX. EFFECTS OF PUNTA TORO VIRUS ON MACROMOLECULAR SYNTHESIS OF CELLS

Introduction

Little has been published about the effects of Punta Toro virus (PTV) on the macromolecular synthesis in cells infected by the virus and its relationship to virus infection. Smith and Pifat (1) have shown that 12 hours after infection assembly of PTV virions can be seen occurring in membranes of the Golgi cisternae. At 24 hours post-infection distinct cytopathic effects were also observed in culture, and cell lysis did not occur until 36 hours after infection. In La Crosse virus infections, the major nucleocapsed protein is first detected at 1-2 hr after infection and remains detectable at 12-15 hr after infection (2). In contrast, the G_1 large glycoprotein was not detected until 4 hr after infection and the G_2 glycoprotein is not detectable until 6 hr after infection. The kinetics is also similar for Rift Valley Fever virus (3). In addition, the members of the *Phlebovirus* group are all thought to inhibit cellular RNA and protein synthesis (4).

The object of this study was to examine the effects of Punta Toro virus on macromolecular synthesis of infected host cells.

Materials and Methods

Virus: The Adames strain of Punta Toro virus (PTV) was propagated as previously described by Sidwell et al. (5).

Cells: A derivative strain of continuously passaged monkey kidney cells (LLC-MK_2), maintained in minimum essential medium (MEM, Grand Island Biological, Grand Island, N.Y.) containing 5% fetal bovine serum (FBS, HyClone Labs, Logan UT) and 0.1% $NaHCO_3$ without antibiotics was used. The cells were determined to be free of mycoplasma.

Effect of PTV infection on log phase and stationary phase LLC-MK_2 cells: LLC-MK_2 cells were seeded in 12 well plates at 1×10^5 cells well and allowed to reach confluence by incubation at 37°C for two days. In another set of experiments cells were seeded at 5×10^4 cells and incubated overnight at 37°C, to obtain log phase cells. Cells were then washed with MEM without serum and PTV virus stock absorbed for 1 hr in each well. Mock infected wells were incubated with MEM without serum. Virus or medium was removed and MEM + 2% serum was added to each well. At various times after virus exposure or mock infection (1, 2, 4, 8, 16, 24, 48 hours for log phase cells and 8, 12, 16, 20 hours for stationary phase cells) media was aspirated and appropriate isotope, diluted in MEM without serum was added to each well. [^{3}H]Leucine was diluted in leucine-deficient MEM. An equal volume of MEM with serum was added for a final concentration of 2% serum. To wells with [^{3}H]leucine, MEM leucine deficient medium + 4% fetal bovine serum was added. Medium was also removed at time 0 from log phase cells, directly after application of virus and cells treated as above. Isotope was incubated for 1 hour at 37°C, removed, and cells fixed with 10% TCA and harvested as previously described by Sidwell, et al. (5). Acid-insoluble CPM were determined in a Packard Scintillation Counter.

Test Statistics: Analysis of variance was used to determine significant differences between log phase and stationary cell experiments. To determine significant differences between time periods for each type of cell, Fisher's LSD test was employed.

Results and Discussion

PTV infection significantly ($P<0.01$) stimulates macromolecular synthesis in log phase cells at 1 hr post-exposure. This time period was not assayed in stationary cells. In contrast virus infection reduced the uptake of [^{3}H]deoxyadenosine into the acid insoluble portions of both stationary phase and log phase cells (Figures IX-1, 2) at 8-24 hours, although more drastically in stationary phase cells. The effects on protein and RNA synthesis appeared to be similar for both PTV infected log- and stationary-phase cells. However, the decrease in uptake of label was significantly more dramatic from 8-24 hours ($P<0.01$) in PTV-infected stationary phase cells.

Interestingly, the inhibition effects of PTV infection on RNA and protein synthesis in LLC-MK_2 cells seemed to be abrogated by 48 hours, although DNA synthesis was still significantly inhibited ($P<0.01$). The depression of macromolecular synthesis at the 16-hour time period in the log phase cell experiment probably represents an aberrant set of wells in which the cells were

not growing very well, since the uptake of a nucleoside of precursors into control cells was 2-5 fold less than that into control cells from other time periods (the level of uptake in control cells remained rather constant for all other time periods, data not shown).

If PTV infection resembles other bunyavirus infections, then from 1-6 hours post-virus exposure, viral proteins and transcripts are being made at optimal amounts (4). The data of this study show that during this time period, the cellular macromolecular processes were initially enhanced and then went back down to normal levels in log phase cells. At 12-24 hours post-virus exposure, other studies have shown that PTV virion assembly begins and cell surface expression of PTV antigens can be detected during this time period (1). The data presented here suggest that during virus assembly the cellular macromolecular synthesis decreased to levels below normal in stationary and log phase cells, in agreement with other studies which show that the *Phlebovirus* group inhibits cellular RNA and protein synthesis. Pifat et al. (1) have also shown that cytopathic effects occur at 36-48 hours post infection in PTV-infected cells. In our study, cellular DNA synthesis became inhibited at that time, while RNA and protein synthesis levels were returned to near normal levels, perhaps reflecting the beginning of cellular death due to viral infection.

Whether the effects described above are an actual stimulation or depression of macromolecular synthesis due to viral induced stimulation or inhibition of cellular enzymes, or viral induced enzymes, or to an increase or decrease in cell permeability to the radiolabeled nucleotide precursors has not been determined.

To better understand these data, they should be correlated with time course studies on the appearance and abundance of viral transcripts and viral proteins within infected cells. It would be useful to determine virus yields from each time period in log phase and stationary cells since the data here indicates that active macromolecular synthesis of the cell may not be necessary for virus production. We should monitor the effects of PTV infection on macromolecular synthesis in stationary phase 0-48 hours to determine if these types of cells are also stimulated during early PTV infection. In addition, studies need to be done to determine the permeability of PTV-infected cells at various times post exposure, to see if the apparent effects on cellular macromolecular synthesis are due to perturbation of those processes or if the uptake is merely a reflection of permeability changes of the cell membrane.

Summary

Punta Toro virus infection appeared to significantly inhibit DNA, RNA and protein synthesis from 16-24 hours post-virus exposure. DNA synthesis, as reflected by deoxyadenosine uptake, remains perturbed throughout PTV infection from 8-48 hours post virus exposure. In addition, PTV seems to enhance macromolecular synthesis 1 hour post exposure to virus in log phase cells. Whether these effects are an actual stimulation or depression of macromolecular synthesis due to viral-induced stimulation or inhibition of cellular enzymes, to viral-induced enzymes, or to an increase or decrease in cell permeability is still to be determined.

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Figure IX-1. Effect of Punta Toro Virus Infection on the Uptake of Radiolabeled Precursors of Macromolecular Synthesis into Stationary Phase LLC-MK_2 Cells.

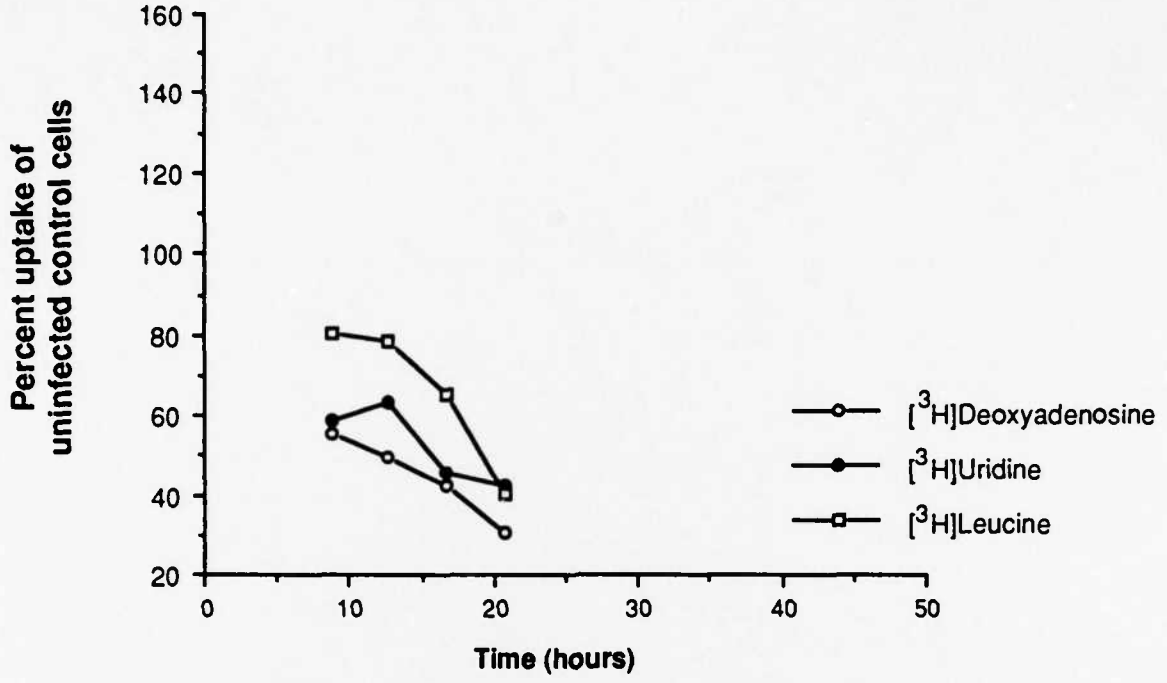
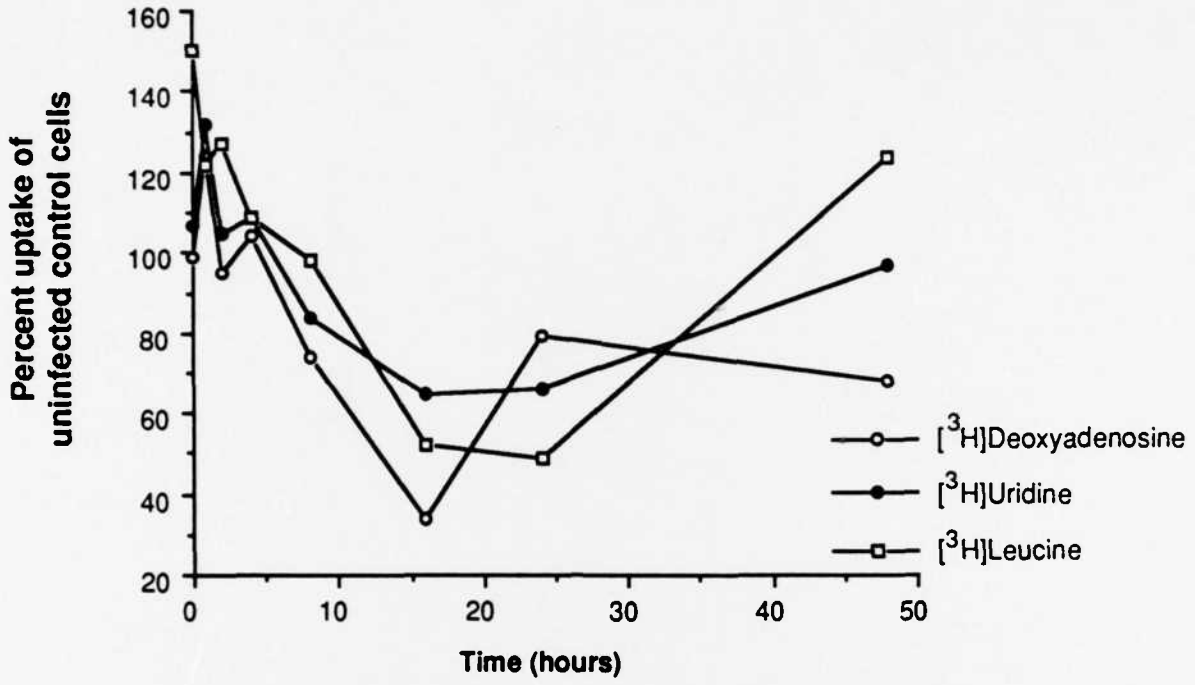


Figure IX-2. Effect of Punta Toro Virus Infection on the Uptake of Radiolabeled Precursors of Macromolecular Synthesis into Log Phase LLC-MK_2 Cells.



X. INVESTIGATIONS INTO THE IN VITRO EFFICACY OF AVS206 AGAINST PUNTA TORO VIRUS

Introduction

AVS206 (ribavirin 3-carboxamidine, ribamidine) has exhibited significant anti-PTV activity both in vitro and in vivo, and has exerted less in vivo toxicity than the known positive drug ribavirin. These observations have prompted a series of studies run to further characterize this compound in cell culture systems.

The studies described here include an in vitro combination study run with AVS206 + AVS01, studies on the effects of various metabolic precursors on the in vitro anti-PTV effects of AVS206, investigations into the deamination of the compound, and biochemical cytotoxicity effects of AVS01 and AVS206.

Materials and Methods

Compounds: Both AVS01 and 206 were provided by Technassociates and later by Biological Research Faculty and Facility, Inc. of Rockville, MD. All metabolic precursors used in reversal experiments were purchased from Sigma Chemical Co. (St. Louis, MO). Radiolabeled materials were obtained from ICN Pharmaceuticals, Inc. (Irvine, CA). Fluorescent silica gel plates (LKGDF, 5 x 20 cm) were purchased from Whatman (Hillsboro, OR).

Virus: The Adames strain of PTV as previously described was used.

Cells: LLC-MK_2 cells (Rhesus monkey kidney) were used as described earlier in Section I.

Results and Discussion

1. Effect of the in vitro combination of AVS01 and AVS206: The methodology for our experiment was essentially identical to that described by Huggins et al. (1), in which combinations of AVS01 and AVS206 in fixed ratios of 10:1, 5:1, 2:1, 1:1, 1:2, 1:5 and 1:10 were evaluated vs PTV. Inhibition of CPE in 96-well microplates was used as criterion for evaluation of activity. Seven concentrations of each drug were used at each ratio, each concentration differing by one-half log from the next. Each compound was also run alone at seven concentrations. ED50's for each drug used alone and each combination of drugs were then determined. The activity was then plotted graphically.

As described by several investigators (2-4), if the line of calculated ED50 values fall below the expected line, the combination is considered synergistic. If the calculated points are above the line the action is indifference; i.e., the drugs do not interact or interfere with each other. Antagonism occurs when the ED50 of either drug is greater in combination than it is alone.

In addition to the graphic technique, we also employed the FIC index method as described in Section VI of this report.

The graphic representation of this combination experiment is seen in Figure X-1. In this figure, the ED50 of AVS01 used alone was 6.5 µg/ml, shown on the y-axis. The ED50 for AVS206 was 20 µg/ml, shown on the x-axis. The line between the two ED50 values indicates where the ED50 values would be expected to fall if the compounds are additive to each other in their antiviral action. The ED50 values for each compound at the designated ratios are also plotted, forming the curve above the line drawn between the ED50's. Since the combination ED50 line is above the expected value line, but the ED50's did not exceed those of AVS01 or AVS206 used alone, we conclude that their combined effect was indifferent or they were interfering with each other.

As seen in Figure X-1, all the FIC values were either additive or in the indifference/partial antagonism range.

Based on these results, we feel AVS01 and AVS206 probably are acting by similar mechanisms, and may be competing somewhat with each other for the same biochemical sites. This possible competition becomes more pronounced as the ratio of AVS206 to AVS01 increases.

2. Effects of metabolic precursors in anti-PTV activity of AVS206: All *in vitro* PTV experiments were performed as described earlier for our routine anti-PTV evaluations, except as noted below.

Initially, the plaque-inhibitory effects of 200, 100, and 50 µg/ml of AVS206 were determined in the presence and absence of 200 µg/ml of adenosine, inosine and guanosine.

In a second series of reversal studies, the effects of adenine, adenosine, cytidine, guanosine, guanosine-5'-PO_4, inosine, thymidine, uridine, and xanthosine were evaluated on PTV *in vitro* and on the anti-PTV effects of AVS206 *in vitro*. In each experiment, 200 µg/ml of the metabolic precursor was used against 1000, 320, 100, 32, 10 and 3.2 µg/ml of AVS206. In this study, inhibition of viral CPE using our standard 96-well microplate system was employed.

A third reversal experiment was run using other related compounds, these being 2'-deoxyadenosine, 2'-deoxycytidine, 2'-deoxyguanosine, hyproxanthine, inosine-5'-PO_4 and orotodine. These compounds were used because previously we also used these materials in reversal studies with ribavirin using other viruses. This third study also used CPE inhibition in 96-well microplates as infectious parameter.

A single reversal study has been run to date using AVS01 (ribavirin) against PTV. Inhibition of CPE was used in this study, with only guanosine and xanthosine used as reversal agents, since these metabolic precursors have previously been found to reverse ribavirin's anti-measles and herpesvirus effects.

The plaque-inhibitory effects of 200, 100 and 50 µg/ml of AVS206 were determined in the presence and absence of 200 µg/ml of adenosine, inosine and guanosine. The results of this experiment are seen in Table X-1. The anti-PTV activity of AVS206 were essentially completely reversed by guanosine. Inosine had no effect. A moderate reversal was seen with adenosine, but an unusual observation was made that adenosine alone, at 200 µg/ml, was inhibitory to PTV-induced plaques.

The results of the second reversal experiment are seen in Table X-2. AVS206 was completely inhibitory to PTV CPE at 1000, 320 and 100 µg/ml; slight CPE was seen at 32 µg/ml. No inhibitory effect was seen at lower dosage levels. Definite reversal of the antiviral activity of AVS206 was seen using adenine, adenosine, guanosine, guanosine-5'-PO_4, and inosine. All of the materials used appear to have a slight reversal on AVS206 antiviral activity at the 320 µg/ml dosage of AVS206; we felt this effect may have been non-specific, so considered only those materials that allowed viral CPE to be seen at 100 µg or higher dose levels of AVS206. It is interesting to note that adenosine, and to a lesser extent, adenine, exhibited a PTV-CPE inhibitory effect when used alone. Both materials also exerted a slight effect on uninfected cells, however; we therefore attribute the antiviral effects of these compounds to a non-specific toxic or static effect.

The results of use of other metabolic precursors as reversing agents with AVS206 are summarized in Table X-3. 2-Deoxyadenosine and 2'-deoxyguanosine were considered reversing agents in this experiment.

The results of the reversal study with AVS01 are seen in Table X-4. Only guanosine and xanthosine were run in this initial study. Guanosine exerted a definite reversal effect, as has been reported previously (5).

At present, considering past reversal experiments run with ribavirin using other RNA viruses, these data suggest that AVS206 may have a similar mechanism of action as ribavirin; i.e., acting as an analogue of guanosine and inhibiting guanosine monophosphate biosynthesis in the infected cell. Ribavirin has previously not been shown to be reversed by adenine, adenosine or 2'-deoxyadenosine; AVS206 does seem to be reversed by these materials. This suggests to us that the compound is also acting as an analogue of adenosine in the cell. It is possible that AVS206 is being partially metabolized to ribavirin in the cell, thus exerting its antiviral effect as both compounds. Our ongoing comparative reversal studies with ribavirin should further determine if this postulate is correct.

3. Effects on uptake of radiolabeled metablic precursors as a measure of cytotoxicity: Experiments were run in 96 well disposable microplates using 4 wells for each drug concentration

and 8 wells for the drug-free control. All drugs and controls were run on the same plate for each radiolabeled precursor.

Approximately 1.5 x 10^5 cells/well in a 24 hr monolayer were incubated with each drug concentration for 3 hr at 37°C followed by a cell wash and a 1 hr pulse with 10 µCi/ml radiolabel in the presence of drug. Fetal bovine serum was absent during the entire period of drug treatment and pulse. Following the pulse period, the medium was aspirated from the cells and 10% sodium dodecyl sulfate (0.1 ml/well) was added. After a 5 min. mild shaking period, 0.1 ml of 20% trichloroacetic acid (TCA) was added and the plates were incubated at 40°C for 2 hr to affect complete precipitation. The precipitate was filtered onto 0.45 µm Millipore filters using 5% TCA for rinse. The dried filters containing the radiolabeled precipitate were then counted on a Packard Scintillation Counter. Percentages of drug-free controls were determined in each dose. These procedures were similar to those described previously by us (6) and by others (7-9).

The results of these studies are summarized in Tables X-5-8. Using [^{3}H]thymidine incorporation, neither ribavirin nor ribamidine were demonstrably inhibitory to cellular DNA synthesis. Using ^{32}P incorporation, ribavirin was inhibitory to DNA synthesis at all concentrations used. AVS206 was inhibitory only at 1000 µg/ml. [^{3}H]Uridine incorporation (RNA synthesis) was not significantly inhibited by any dose of ribavirin, and only by the 1000 and 100 µg/ml dosages of the carboxamidine. These ribavirin data showing an effect on RNA synthesis do not match previous data we have reported with this compound using MA-104 cells (6) and may be due to the cell differences. Such a variation in ribavirin's effects due to cell differences have been reported previously (10). Both compounds were considered to have moderate effects on protein synthesis (Table X-4), although these effects were not dose-responsive.

The data with ribavirin regarding inhibition of protein synthesis correlate well with those previously reported (6). This latter effect may be a general effect on amino acid transport into the cell, rather than direct inhibition of protein synthesis. These observations suggest both compounds to have a static, rather than a toxic effect on the cell. Of importance in these assays was the use of an established and confluent cell monolayer in contrast to rapidly dividing cells often used in such studies (7, 8). Such cell monolayers, exposed for a relatively long period of time to the test compounds prior to pulsing, were used to more closely duplicate the conditions of the antiviral experiments.

4. Investigations into the deamination of AVS206: Compounds AVS01 (ribavirin) and AVS206 (ribavirin 3-carboxamidine, ribamidine) are almost identical, with the exception that the carboxamide group of AVS01 is converted to a carboxamidine group to give AVS206. AVS206 is thought to be merely deaminated to AVS01 *in vitro* and *in vivo*, presumably *in vivo* by host deaminases. In addition, AVS206 in aqueous solution is also known to hydrolyze to AVS01, especially upon prolonged storage (R.K. Robins, ICN Pharmaceuticals, *personal communication*). This study will provide evidence that AVS206, after being treated with adenosine deaminase, is altered to a compound that comigrates with AVS01 on silica gel thin lay chromatography plates.

Nucleosides and analogs were suspended in 10 mM phosphate buffer, pH 6.5. Each compound was incubated with adenosine deaminase (50:1, compound to enzyme) at 25°C for 1 hr.

Five microliter samples were directly applied to silica gel plates without stopping the deaminase reaction and dried at room temperature for 15 minutes. Plates were developed with 2-propanol-aqueous ammonia (fresh)-H_2O (7:1:2). Using ascending chromatography, plates were developed for about 3 hours until solvent front migrated 15 cm. Plates were allowed to air dry overnight to eliminate any traces of free ammonia.

Plates were placed in a 2000 ml beaker layered with a 0.5 cm layer of $CaHClO_3$, covered, and incubated for 2 min. The plates were then transferred to another 2000 ml beaker and placed down in the large beaker beside a smaller beaker filled with 20 ml of formalin. The larger beaker was covered and the plates were exposed to the formalin for 45 seconds. The plates were removed and immediately sprayed with a fine mist of 1% soluble potato starch, 1% potassium iodide, and 0.05% Triton X-100 (freshly made). The N-chlorinated nucleosides or analogs were detected as dark purplish-black spots. Migration distances of each compound were measured relative to the solvent front in each track.

A variety of compounds can be deaminated by adenosine deaminase, including adenosine, guanosine, 2,6-diaminopurine(2'-deoxy)riboside, and 6-methoxypurine. Therefore, adenosine deaminase was chosen as the likely enzyme that could deaminate AVS206, because of the relative lack of specificity of the enzyme.

When treated with adenosine deaminase, the migration of AVS01 was unaffected and adenosine, as expected, was converted to inosine as well as other compounds (Table X-9, Figure X-2). Guanosine did not deaminate. However, AVS206 was converted to two migrating species, one that comigrated with AVS01 and another species that presented itself as a broad, smeared, band. The species with an R_f value of 71 may have been ribavirin. The smeared band probably represents several compounds including AVS206. Some of these compounds could be stable enzymatically-derived intermediates or perhaps a final end product such as an NH_2OH derivative. Further support for this hypothesis is the fact that when AVS206 was stored for a prolonged time period and consequently hydrolyzed non-enzymatically, only two discrete bands appeared after chromatography (Figure X-2). Smeared bands only appeared when the substrate was susceptible to deaminase cleavage. Contaminants due to buffer or water were also not contributors to the smearing (Table X-9). Smearing due to sample overload can also be eliminated as a factor, since preparations without enzyme did not smear as seen in Table X-9. Contaminating enzymes in the deaminase preparation which could also generate different species of compounds were not important due to their low concentration in the enzyme preparation (5'-AMP deaminase,<0.002%; alkaline phosphotase,0.004%; guanase, <0.001%; nucleoside phosphorylase, 0.005%; percentage of adenosine deaminase activity).

Since adenosine deaminase is a common serum deaminase and also a major contaminant of fetal bovine serum, AVS206 could very well be deaminated in *in vitro* and *in vivo* to AVS01 by this enzyme. But, this does not explain the different efficacies of AVS206 and AVS01 against PTV *in vivo* as we have reported (11). However, the possibility exists that some stable intermediate form of AVS206 (perhaps a species found in the smeared band R_f=55-65) may be generated by deaminase activity that could provide the striking anti-PTV activity of AVS206. It is also possible that the compound is acting as a prodrug for ribavirin.

In a separate experiment, AVS206, dissolved in MEM supplemented with 0.1% $NaHCO_3$, was stored at 5°C for 2 weeks, after which it was evaluated as above for chromatographic migration. A definite separation of a portion of the compound to AVS01 was seen (Table X-9), indicating that the compound does indeed hydrolyze to a compound which we presume to be ribavirin upon prolonged storage.

Conclusions

1. AVS01 and AVS206, when used in combination vs Adames PTV in an *in vitro* experiment, appeared to have an indifferent or partial antagonistic effect. These data suggest the compounds are probably acting by similar mechanisms and may be competing with each other.

2. The *in vitro* anti-PTV activity of AVS206 was reversed by adenosine, 2-deoxyadenosine, guanosine, guanosine 5'-PO_4, and 2-deoxyguanosine. Other precursors, including inosine, adenine, cytidine, thymidine, uridine, and xanthosine did not have a noticeable reversal effect. The anti-PTV activity of AVS01 was reversed by guanosine but not by xanthosine.

3. Neither AVS206 nor AVS01 were considered strongly cytotoxic as measured by effects on DNA, RNA, and protein synthesis. AVS206 was less inhibitory to DNA synthesis as measured by uptake of ^{32}P than AVS01. Our data suggest both materials to have a static, rather than toxic, effect on LLC-MK_2 cells.

4. AVS206 and AVS01 were subjected to enzymatic degradation with adenosine deaminase. AVS206 was broken down to a species that comigrated with AVS01 as determined by silica gel thin layer chromatography. AVS01 was unaffected by deaminase treatment. In addition, with prolonged incubation, AVS206 apparently degenerates to a species that also comigrates with AVS01.

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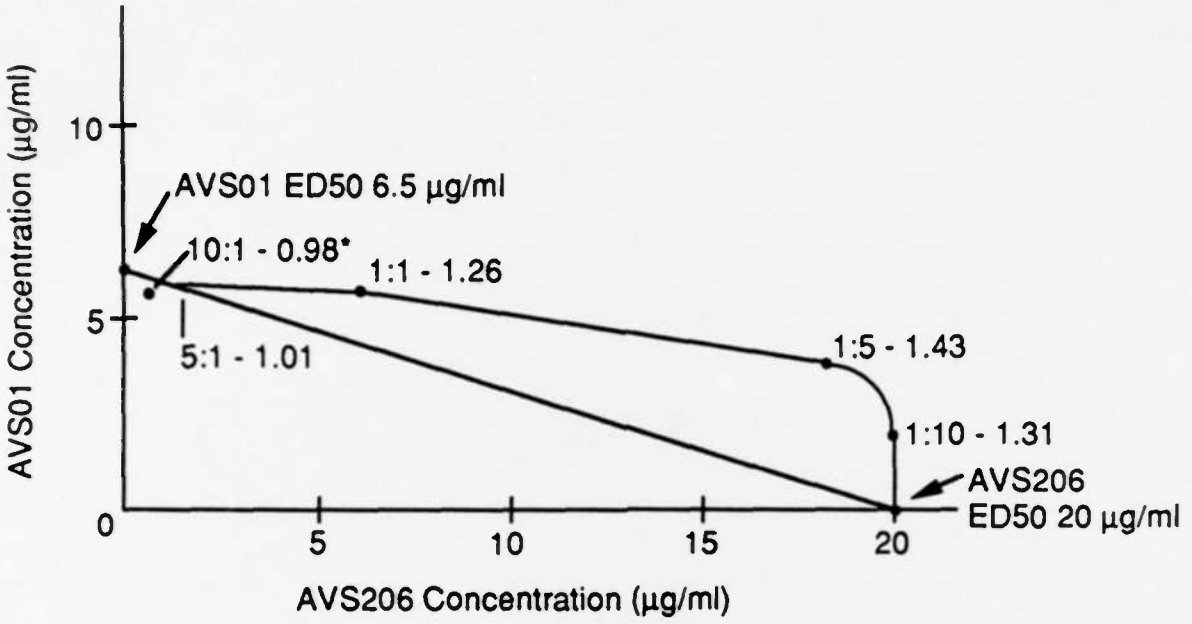
Figure X-1. PTC653-8. Comparison of Effects of Ribavirin (AVS01) and Ribavirin Carboxamidine (AVS206) Used Alone and in Combination on Punta Toro Virus Infections in LLC-MK_2 Cells.



*Ratio - FIC Index

Table X-1. PTC666-669. Reversal of AVS206 Punta Toro Virus Plaque Inhibition by Adenosine, Guanosine, or Inosine[a].

Concentration AVS206 (µg/ml)	Avg. No. Plaques/Well			
	No Additive	Adenosine (200 µg/ml)	Inosine (200 µg/ml)	Guanosine (200 µg/ml)
200	0	0	0	48
100	0	17	0	58
50	35	12	42	68
0	68	0	74	76

[a]Plaques induced in LLC-MK_2 cells. Metabolites added concomitantly with AVS206 to infected cells.

Table X-2. PTC641-650. Reversal of AVS206 Anti-Punta Toro Virus Activity by Various Purines, Nucleosides or Nucleotides[a].

Concentration AVS206 (µg/ml)	Average CPE Score[b] @ Additive Concentration Shown									
	No Additive	Adenine (200 µg/ml)*	Adenosine (200 µg/ml)*	Cytidine (200 µg/ml)	Guanosine (200 µg/ml)	Guanosine 5'-PO_4 (200 µg/ml)	Inosine (200 µg/ml)	Thymidine (200 µg/ml)	Uridine (200 µg/ml)	Xanthosine (200 µg/ml)
1000*	0	0	0	0	0	0	0	0	0	0
320*	0	0	0.2	0	0.5	0.5	0	0	0	0
100*	0	0.5	1.0	0	2.0	2.7	0.3	0	0	0
32	0.7	1.7	3.2	1.3	3.5	4.0	4.0	3.5	3.7	2.0
10	4.0	3.0	1.3	4.0	4.0	3.8	4.0	4.0	4.0	4.0
3.2	4.0	2.7	1.2	4.0	4.0	4.0	4.0	4.0	4.0	4.0
0	4.0	2.7	0.7	4.0	4.0	4.0	4.0	4.0	4.0	4.0

[a]CPE induced in LLC-MK_2 cells. Each compound added concomitantly.

[b]CPE scored from 0 (normal cells) to 4 (maximal cell destruction).

*Slight cytotoxicity also seen using these materials.

Table X-3. PTC670-676. Further Studies on the Reversal of AVS206 Anti-Punta Toro Virus Activity by Various Compounds[a].

Concentraion AVS206 (µg/ml)	Average CPE Score[b] @ Additive Concentration Shown						
	No Additive	2'-Deoxy-adenosine (200 µg/ml)*	2'-Deoxy-cytidine•HCl (200 µg/ml)	2'-Deoxy-guanosine (200 µg/ml)	Hypoxan-thine (200 µg/ml)	Inosine 5'-phosphate Na salt (200 µg/ml)	Orotidine (200 µg/ml)
1000*	0	0	0	0	0	0	0
320*	0	0.2	0	0.5	0	0	0
100*	0	1.8	0.3	2.7	0.3	0.3	0.3
32*	2.8	4.0	3.7	3.8	3.8	4.0	3.5
10	4.0	4.0	4.0	4.0	4.0	4.0	4.0
3.2	4.0	4.0	4.0	4.0	4.0	4.0	4.0
0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

[a]CPE induced in LLC-MK_2 cells. Each compound added concomitantly.

[b]CPE scored from 0 (normal cells) to 4 (maximal cell destruction).

*Slight cytotoxicity also seen using these materials.

Table X-4. PTC651-653. Reversal of AVS01 Antiviral Activity by Guanosine or Xanthosine[a].

Concentration AVS01 (µg/ml)	Average CPE Score[b] @ Additive Concentration Shown		
	No Additive	Guanosine (200 µg/ml)	Xanthosine (200 µg/ml)
1000	0	0	0
320	0	0	0
100	0	0.5	0
32	0.5	2.3	0
10	2.0	3.7	0.8
3.2	3.5	3.8	4.0
0	4.0	4.0	4.0

[a]CPE induced in LLC-MK_2 cells. Each compound added concomitantly.

[b]CPE scored from 0 (normal cells) to 4 (maximal cell destruction).

Table X-5. Effects of AVS01 and AVS206 on [^{3}H]Thymidine Incorporation into LLC-MK$_2$ Cells[a]

Compound	Drug Concentration (µg/ml)	[^{3}H]Thymidine Incorporation	
		CPM[b]	% of Control ± SE[c]
AVS01	1000	24,993	105 ± 20
	100	21,886	91 ± 5
	10	22,884	95 ± 9
	1	27,434	115 ± 7
	0.1	27,434	115 ± 4
	0	23,887	100 ± 5
AVS206	1000	22,535	90 ± 18
	100	24,522	102 ± 10
	10	32,617	137 ± 5
	1	31,056	130 ± 10
	0.1	31,753	133 ± 8
	0	23,887	100 ± 5

[a]24 hr monolayer initially, then incubated with drug for 23 hr, followed by 1 hr radiolabelled pulse.

[b]Mean of four replicates.

[c]Standard Error of percentage.

Table X-6. Effects of AVS01 and AVS206 on Inorganic [^{32}P] Incorporation into LLC-MK$_2$ Cells[a]

Compound	Drug Concentration (µg/ml)	[^{32}P] Incorporation CPM[b]	[^{32}P] Incorporation % of Control ± SE[c]
AVS01	1000	2,955	50 ± 7
	100	2,205	37 ± 6
	10	3,643	59 ± 4
	1	3,498	62 ± 8
	0.1	3,754	63 ± 5
	0	5,924	100 ± 16
AVS206	1000	3,677	62 ± 10
	100	6,486	110 ± 3
	10	6,199	104 ± 3
	1	7,317	124 ± 2
	0.1	10,888	184 ± 6
	0	5,924	100 ± 16

[a]24 hr monolayer initially, then incubated with drug for 23 hr, followed by 1 hr radiolabelled pulse.

[b]Mean of four replicates.

[c]Standard Error of percentage.

Table X-7. Effects of AVS01 and AVS206 on [^{3}H]Uridine Incorporation in LLC-MK$_2$ Cells[a]

Compound	Drug Concentration (µg/ml)	[^{3}H]Uridine Incorporation	
		CPM[a]	% of Control ± SE[b]
AVS01	1000	113,888	105 ± 7
	100	99,257	89 ± 6
	10	98,207	88 ± 6
	1	137,961	125 ± 8
	0.1	128,426	116 ± 15
	0	110,617	100 ± 7
AVS206	1000	49,489	44 ± 8
	100	46,552	42 ± 5
	10	130,636	116 ± 2
	1	121,897	102 ± 7
	0.1	127,403	115 ± 5
	0	110,617	100 ± 7

[a]24 hr monolayer initially, then incubated with drug for 23 hr, followed by 1 hr radiolabelled pulse.

[b]Mean of four replicates.

[c]Standard Error of percentage.

Table X-8. Effects of AVS01 and AVS206 on [^{3}H]Leucine Incorporation into LLC-MK$_2$ Cells[a]

Compound	Drug Concentration (µg/ml)	[^{3}H]Leucine Incorporation	
		CPM[b]	% of Control ± SE[c]
AVS01	1000	91,223	52 ± 6
	100	113,893	64 ± 2
	10	131,734	75 ± 4
	1	116,979	67 ± 5
	0.1	131,497	75 ± 4
	0	175,252	100 ± 7
AVS206	1000	77,394	44 ± 4
	100	113,340	65 ± 10
	10	107,411	61 ± 12
	1	112,634	64 ± 12
	0.1	119,139	68 ± 10
	0	175,252	100 ± 7

[a]24 hr monolayer initially, then incubated with drug for 23 hr, followed by 1 hr radiolabelled pulse.

[b]Mean of four replicates.

[c]Standard Error of percentage.

Figure X-2. The effects of deaminase treatment of selected nucleosides and nucleoside analogs.

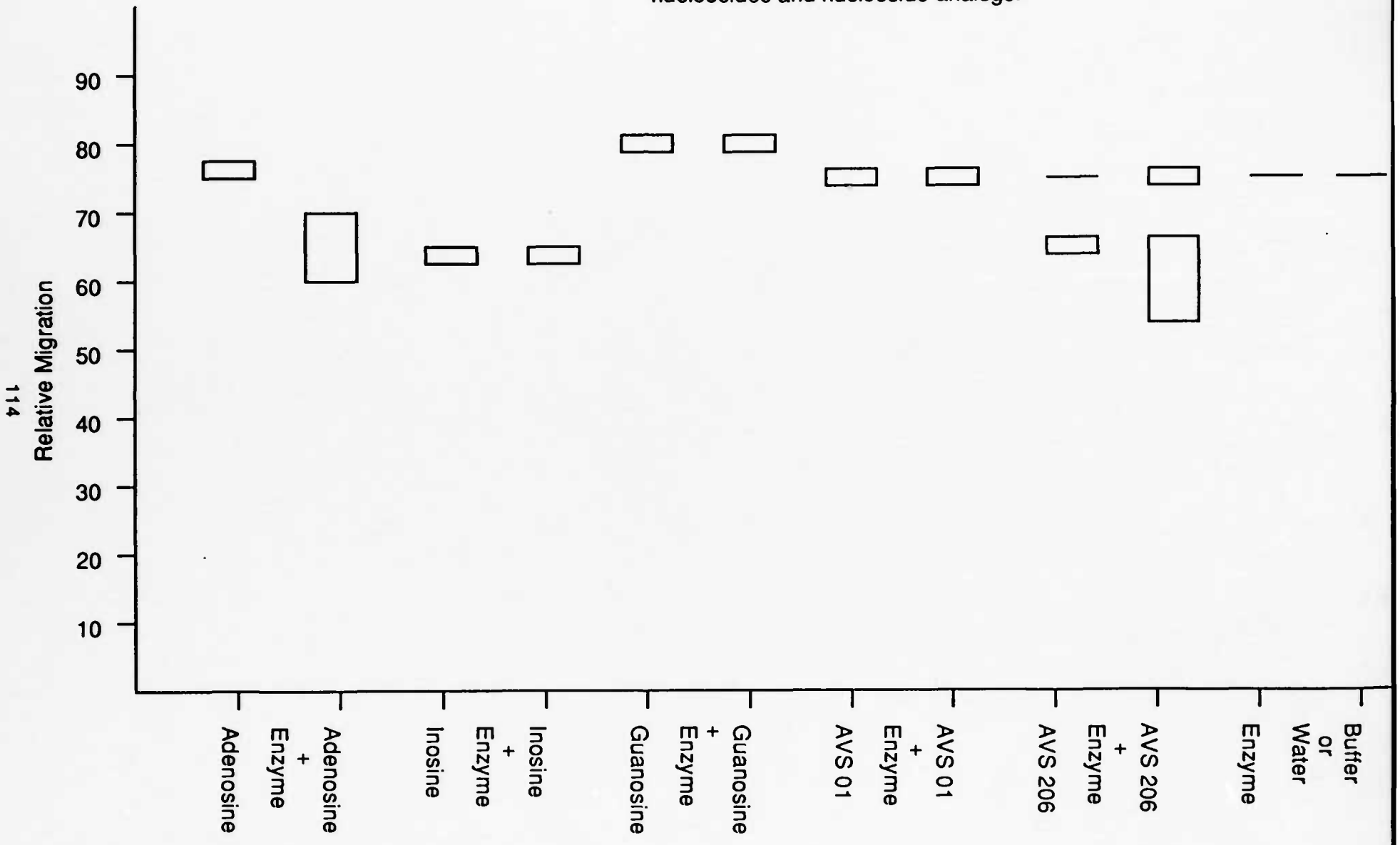


Table X-9. The Effects of Deaminase Treatment of Selected Nucleosides and Nucleoside Analogs.

Compound	Relative Migration Distance (R_f)[a]	
	Untreated	Deaminase Treated
AVS 01	72	72
AVS 206	63,72[b]	71,55-65[bc]
Adenosine	75	60-70[c]
Inosine	65	65
Guanosine	75	75
Buffer[d]	75	75
AVS 206[e]	63,72[b]	ND

[a]All Distances were measured relative to solvent front using a template spotting guide.

[b]Minor band.

[c]Band was smeared

[d]Buffer with or without enzyme, no nucleoside added.

[e]AVS 206 was incubated in MEM supplemented with 0.1% sodium bicarbonate at 5°C for 2 weeks and then sampled.

XI. EFFECTS OF TREATMENT WITH AVS206 ON DELAYED INFECTION PARAMETERS IN PUNTA TORO VIRUS-INFECTED MICE

Introduction

AVS206 (ribavirin 3-carboxamidine, ribamidine) has exhibited significant anti-PTV activity in vitro and in vivo. The purpose of the present study was to determine if the antiviral effects rendered by this compound are still seen when the animals were assayed at later times in the infection.

Materials and Methods

Compound: AVS206 was provided by Technassociates, Inc for this study. The compound was dissolved in sterile water for this study.

Virus: The Adames strain of PTV as previously described was used.

Animals: Female 3 week-old C57BL/6 mice (Simonsen) as described previously were used after a 24 hr quarantine.

Experiment Design: Mice were infected s.c. with a standard inoculum of PTV, then treated p.o. twice daily for 3 days with 1000 mg/kg/day of AVS206 beginning 24 hr post-virus inoculation. Five infected, AVS206-treated and H_2O-treated mice were then killed on infection days 3, 4, 5, 6, and 7. The livers were given scores of 0 to 4 for hepatic icterus and the livers and sera were individually assayed for virus titer by our standard procedure using LLC-MK_2 cells. Mice dying before sacrifice were assumed to have 4+ liver scores and maximal liver and serum virus titers.

All assays for virus were as described for our standard *in vivo* anti-PTV experiments

Results and Discussion

The results of this study are seen in Table XI-1. On day 3, all livers from mice treated with AVS206 appeared normal and no virus was recovered from their livers or sera. The H_2O-treated infected animals had a relatively low liver score but high virus titers in both livers and sera. By day 4, the virus control mice had markedly discolored livers and high titers of virus recovered from livers and sera. The AVS206-treated mice had slight (mean 0.4) liver discoloration and low ($10^{0.5}$) virus in their liver but none in their serum. The liver scores of the AVS206-treated mice declined to 0 through the remainder of the experiment, although moderate (less then $10^{2.0}$) virus levels were seen in the livers and sera from the mice on day 5. No virus was seen in livers or serum samples from these animals on days 6 and 7. All of the virus control mice had died by day 5.

We conclude that treatment with AVS206 was highly effective in reducing the infection in the mice, and, once treatment terminated by the end of day 3, the infection did not return to any significant extent.

Conclusions

AVS206 administered p.o. twice daily for 3 days was highly effective vs Adames PTV infections in mice, and, once treatment was terminated, detectable infectious virus did not return to either livers or sera from surviving mice up to 4 days later.

Table XI-1. Expt. PtA447. Effect of AVS206 on Punta Toro Virus Infections in Mice When Assayed at Varying Days Post-Virus Inoculation.

Animals: 11.6-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.
Treatment Schedule: Twice daily x 3, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Compound	Experiment Day[a]	Mean Liver Score[b]	Mean Liver Virus Titer (log_{10})	Mean Serum Virus Titer (log_{10})
AVS206[c]	3	0.0**	0.0**	0.0**
H_2O		1.2	5.4	6.2
AVS206	4	0.4**	0.5**	0.0**
H_2O		3.1	4.0	5.0
AVS206	5	0.0**	1.8**	1.1**
H_2O[d]		4.0	5.7	6.5
AVS206	6	0.0**	0.0**	0.7**
H_2O		4.0	5.7	6.5
AVS206	7	0.0**	0.0**	0.0**
H_2O		4.0	5.7	6.5

[a]Days post-virus inoculation on which five animals were sacrificed.

[b]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day of sacrifice (animals dying prior to sacrifice assigned a liver score of 4).

[c]AVS206 concentration was 1000 mg/kg/day. Toxicity controls all survived and gained 0.6 g as compared to 1.2 g in normal controls.

[d]All H_2O controls were dead by day 5 of experiment, therefore all animals were given the highest score observed before this time.

**$P<0.01$ as compared with the corresponding H_2O control animals.

Conclusions: Ribamidine had previously been shown to markedly affect PTV infections, with inhibition of serum and liver virus titers and mean liver score seen at 2 or 3 days post-virus inoculation. The present study was run to determine if this therapy maintained a low score and virus titer if assayed at later times. The data indicate slight rises in virus titer by day 5, but this subsided again by days 6 and 7.

XII. COMPARISONS OF THE BIOCHEMICAL CYTOTOXICITY OF AVS01, 111, 253, 257, AND 3706

Introduction

The compounds ribavirin (AVS01), tiazofurin (AVS111), selenazofurin (AVS253), tiazofurin 5'-monophosphate (AVS257), and tiazofurin triacetate (AVS3706) have all exhibited significant *in vitro* and *in vivo* activity against PTV. As a means of further determining the true potential of these compounds as possible therapies for infections of this type, we have run experiments to compare their biochemical cytotoxicities in LLC-MK_2 cells. This section describes the results of these studies.

Materials and Methods

Cells: LLC-MK_2 (Rhesus monkey kidney) cells were used. They were obtained initially from the ATCC. Various passages of the cells were used over the 1-year period of this study. Growth medium was medium essential medium (MEM, GIBCO Labs, Grand Island, NY) containing 5% fetal bovine serum (FBS, HyClone Labs, Logan, UT) and 0.1% $NaHCO_3$ without antibiotics. All were determined to be mycoplasma-free.

Test Compounds: All materials were provided by Biological Research Faculty & Facility (BRFF) for these tests. Each was stored and handled according to instructions from BRFF.

Cytotoxicity Determinations Using Radiolabel Uptake: The cytotoxicity assay was performed as described by Smee, et al (1). Briefly, 24 h confluent cell monolayers in 96 well plates were incubated with medium containing 5% serum and the appropriate concentration of drug for 24 hours at 37°C. Four different concentrations of drugs (including a control without drug) were tested with each run in four replicate wells. After 24 h, the medium was removed and the cells were pulsed for 1 h with 10 µCi/ml of radioactive isotope ([^{3}H]thymidine, [^{3}H]uridine, [^{3}H]leucine) and fresh drug. In [^{3}H]leucine experiments, the 1 h pulse with label was done in medium devoid of nonradioactive leucine. After incubation for 1 hour at 37°C, all wells were aspirated to dryness. The cells were fixed with 5% trichloroacetic acid (TCA) for 1 hour at 4°C. The plates were gently tapped for 30 seconds and 75 µl from each well was removed and placed in scintillation vials to count acid-soluble radioactivity. The remainder of the TCA was removed from the wells and discarded. The precipitate at the bottom of each well was redissolved in 0.2 ml of 0.5N KOH and incubated for 1-2 hours at 37°C. The entire contents of the wells were placed in scintillation vials to count acid-insoluble radioactivity. An overview of this procedure is seen in Figure XII-1.

Experiment Design: In these studies, one set of experiments was run to compare the activities of AVS111 and AVS3706 to that of AVS01. In the second study, AVS253 and AVS257 were compared to AVS01.

Results and Discussion

The results of the experiments with AVS111, 3706, and 01 are summarized in Figure XII-2 (RNA synthesis inhibition), Figure XII-3 (DNA synthesis inhibition), and Figure XII-4 (protein synthesis inhibition). Both tiazofurin and its triacetate were more cytotoxic than ribavirin as measured by all three assays. This was particularly evident in the inhibition of RNA and protein synthesis (Figures XII-2 and XII-4). Ribavirin's cytotoxicity was essentially as we have seen previously (1).

The results of the study using AVS253, 257, and 01 are seen in Figures XII-5 (RNA synthesis inhibition), Figure XII-6 (DNA synthesis inhibition), and Figure XII-7 (protein synthesis inhibition). Ribavirin was least cytotoxic, followed by selenazofurin and then tiazofurin 5'-monophosphate. At the lower doses using inhibition of protein synthesis (Figure XII-7), AVS257 was somewhat more toxic than either of the two other compounds.

These data indicate that, although these ribavirin analogs have significant anti-PTV effects *in vitro* and *in vivo*, they have a considerable degree more cytotoxicity than the parent compound and are thus probably not wholly suitable as replacements for ribavirin.

Conclusions

AVS111, 253, 257, and 3706 were considered more cytotoxic to LLC-MK2 cells than AVS01 as measured by effects on DNA, RNA, and protein synthesis.

Literature Cited

1. Smee, D.F., J.C. Martin, J.H. Verheyden, and T.R. Matthews. (1983) Antiherpesvirus activity of the acyclic nucleoside 9-(1,3-dihydrowy-2-proposymethyl)guanine. Antimicrob. Ag. Chemother. 23:676-682.

2. Barnard, D. L. and R. W. Sidwell. 1989. The effects of a *Phlebovirus* inhibitory agent, ribamidine, on cell proliferation and macromolecular synthesis in LLC-MK_2 cells. Abst. Ann. Mtg. Amer. Soc. Microbiol. T-41.

Figure XII-1. Scheme For Determining Cytotoxic Effects On Cellular Macromolecular Synthesis

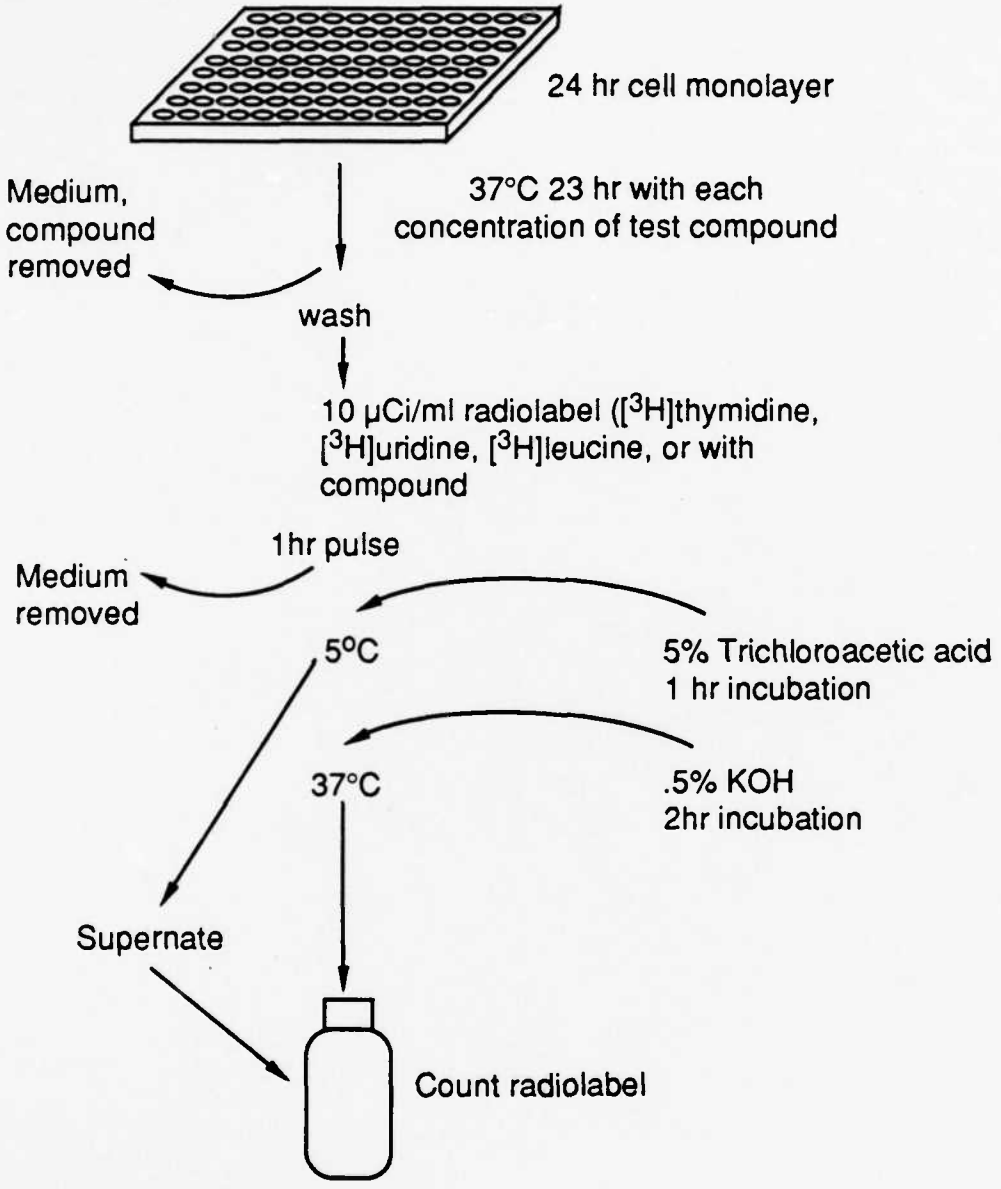
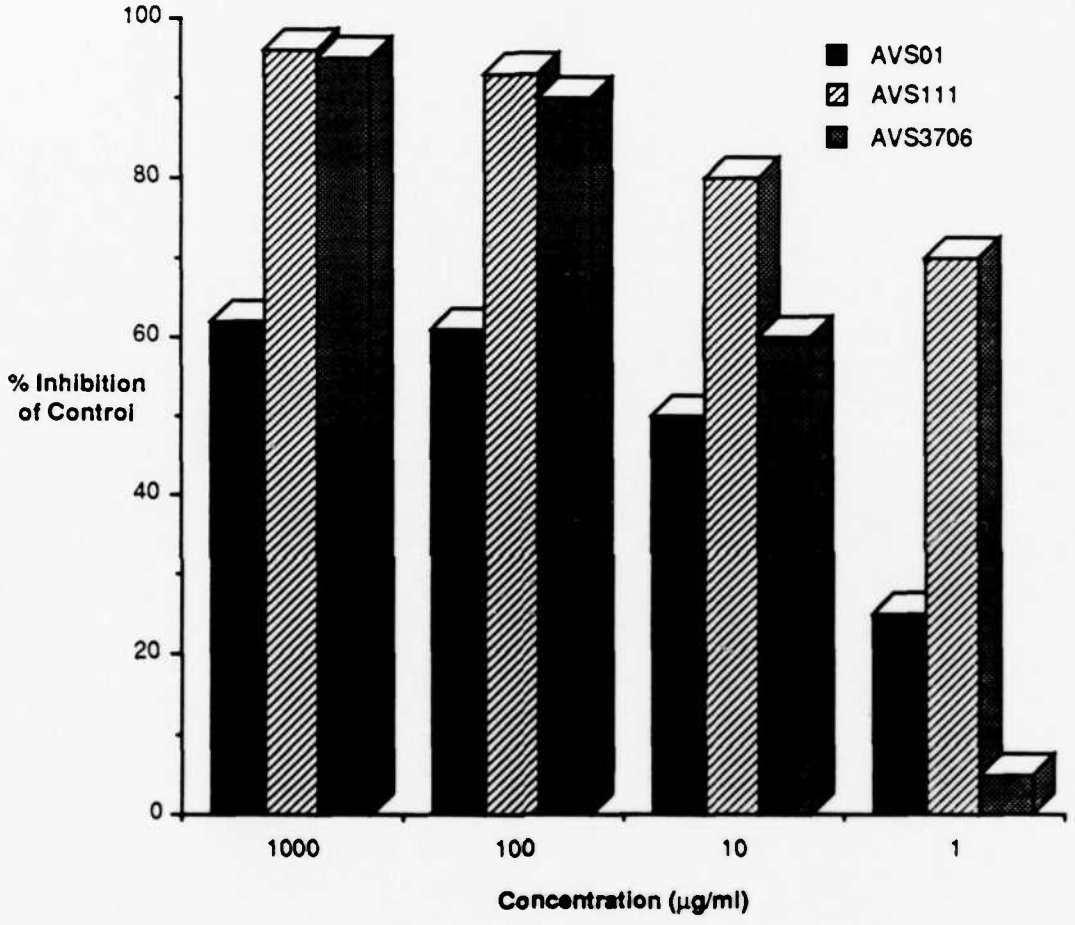
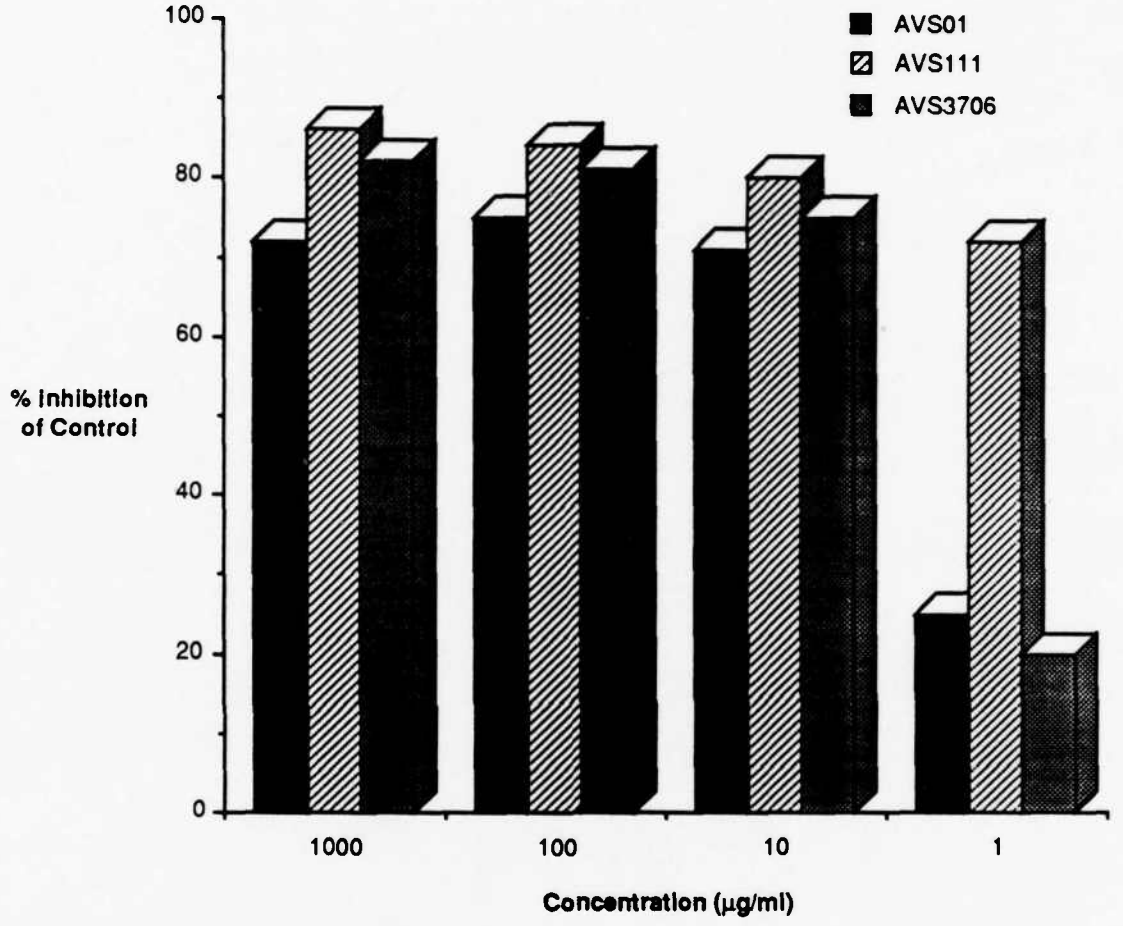


Figure XII-2. Comparison of the Cytotoxicity of (RNA Synthesis Inhibition[a]) of AVS01 (Ribavirin), AVS111 (Tiazofurin) and AVS3706 (Tiazofurin Triacetate) in LLC-MK_2 Cells.



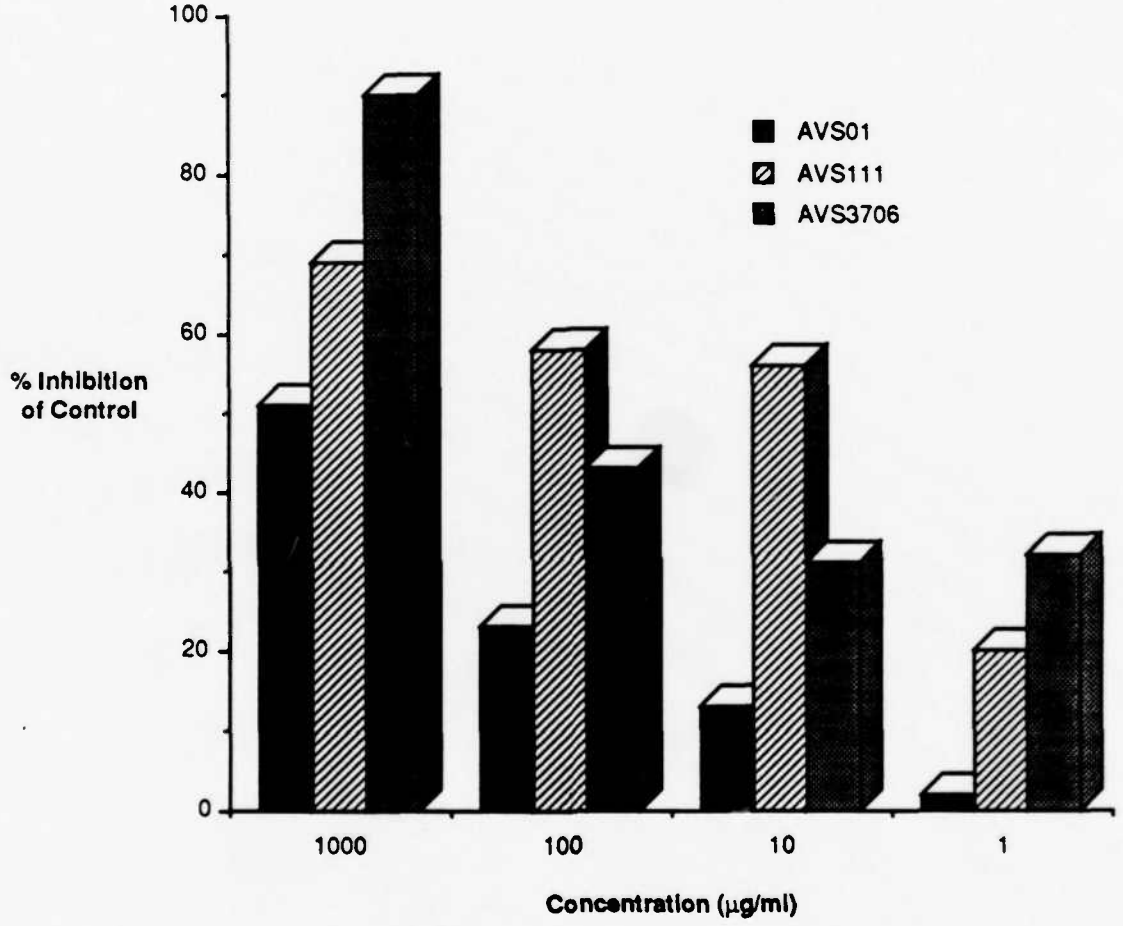
[a] Assayed by [^{3}H]Uridine uptake.

Figure XII-3. Comparison of the Cytotoxicity of (DNA Synthesis Inhibition [a]) of AVS01 (Ribavirin), AVS111 (Tiazofurin) and AVS3706 (Tiazofurin Triacetate) in LLC-MK_2 Cells.



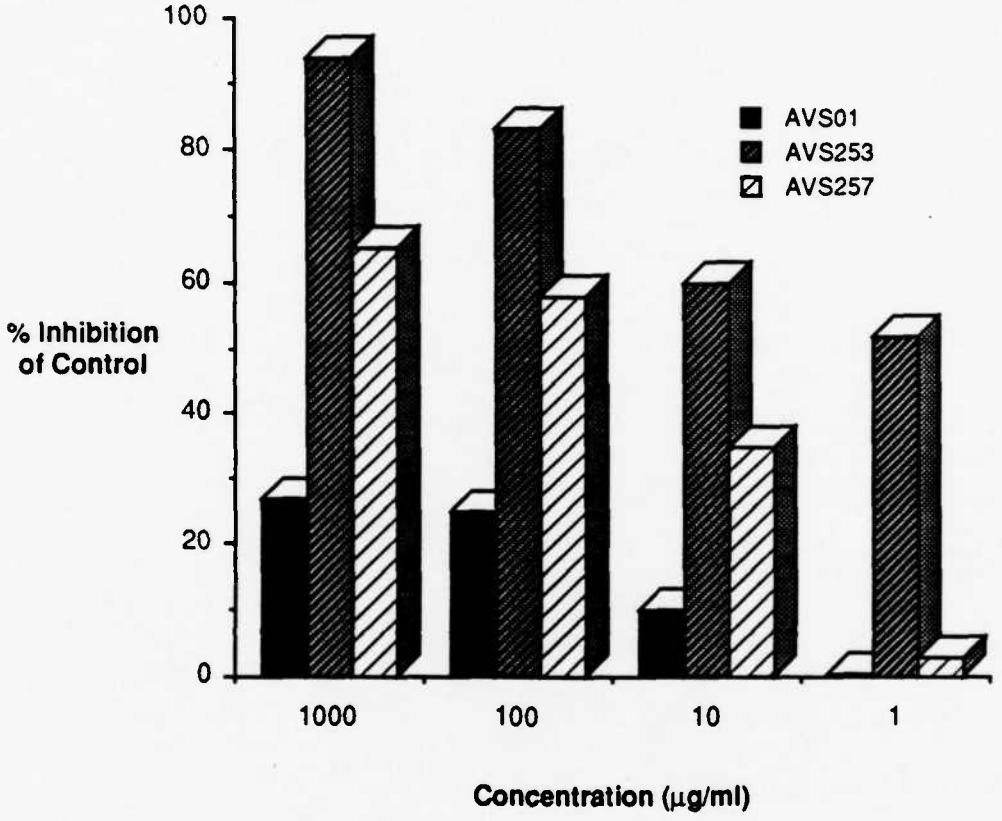
[a] Assayed by [^{3}H]thymidine uptake.

Figure XII-4. Comparison of the Cytotoxicity of (Protein Synthesis Inhibition[a]) of AVS01 (Ribavirin), AVS111 (Tiazofurin) and AVS3706 (Tiazofurin Triacetate) in LLC-MK$_2$ Cells.



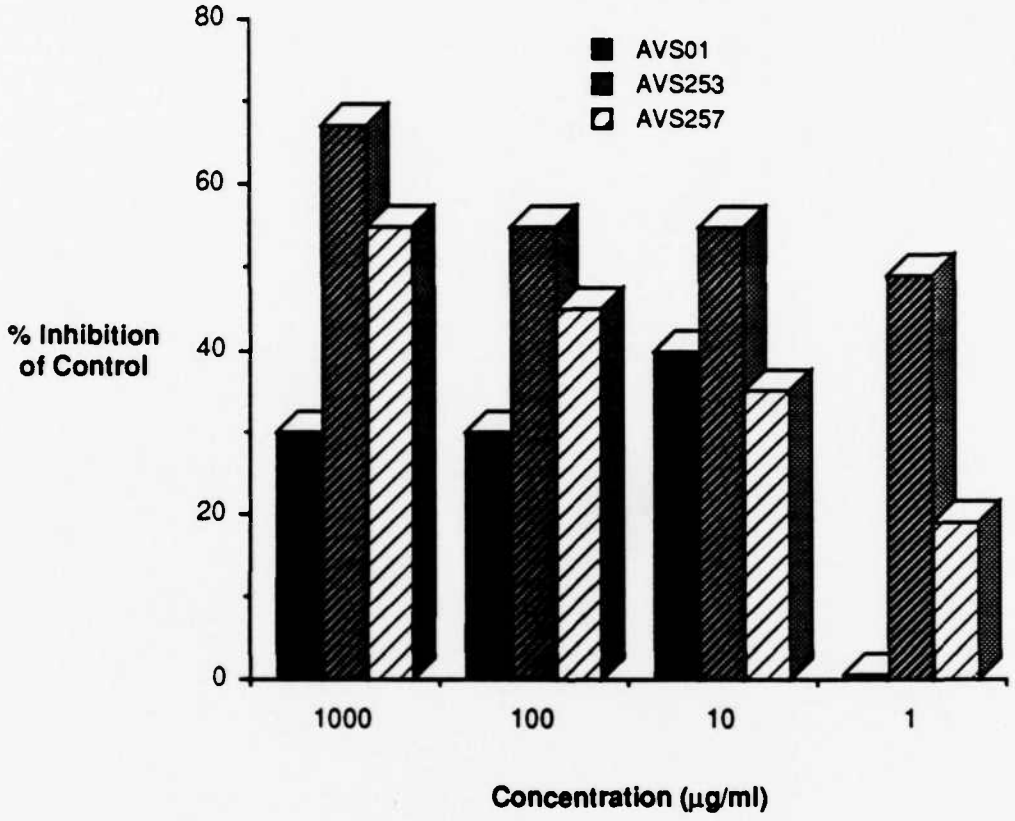
[a] Assayed by [^{3}H]Uridine uptake.

Figure XII-5. Comparison of the Cytotoxicity (RNA Synthesis[a]) of AVS01 (Ribavirin), AVS253 (Selenazofurin), and AVS257 (Tiazofurin 5'-monophosphate) in LLC-MK_2 Cells.



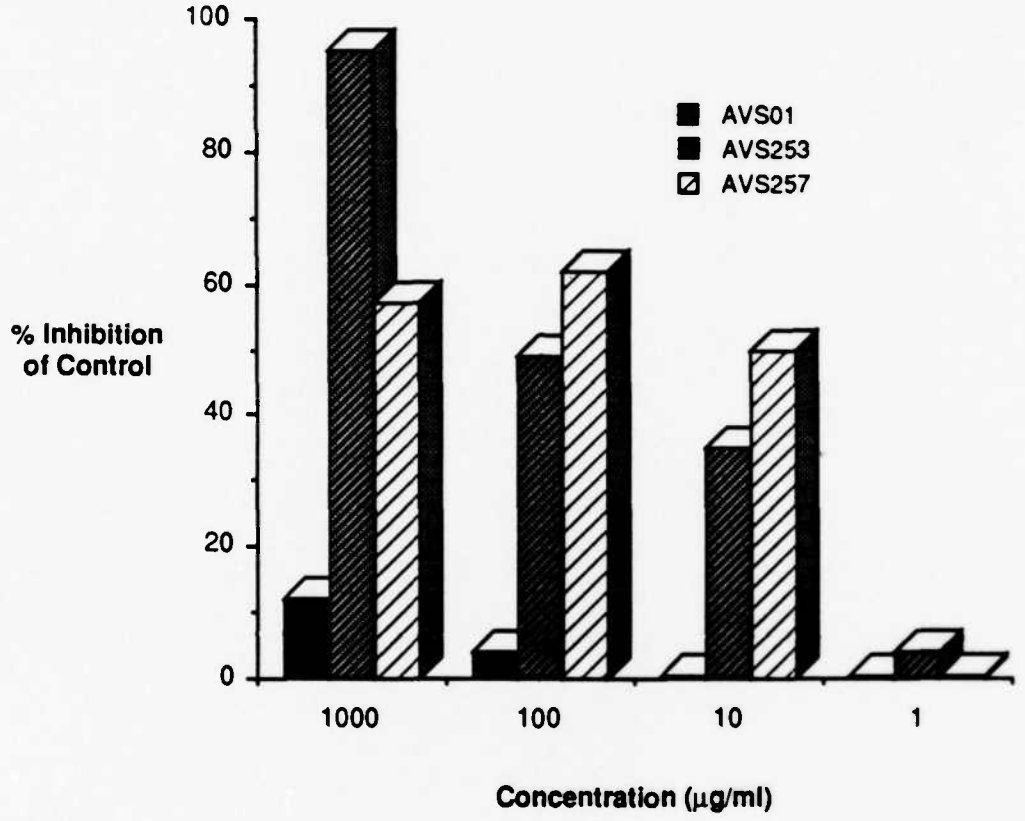
[a] Assayed by [^{3}H]uridine uptake.

Figure XII-6. Comparison of the Cytotoxicity (DNA Synthesis[a]) of AVS01 (Ribavirin), AVS253 (Selenazofurin), and AVS257 (Tiazofurin 5'-monophosphate) in LLC-MK_2 Cells.



[a] Assayed by [^{3}H]thymidine uptake.

Figure XII-7. Comparison of the Cytotoxicity (Protein Synthesis[a]) of AVS01 (Ribavirin), AVS253 (Selenazofurin), and AVS257 (Tiazofurin 5'-monophosphate) in LLC-MK_2 Cells.



[a] Assayed by [^{3}H]leucine uptake.

XIII. A MEASUREMENT OF AVS01 TOXICITY USING PULSE OXIMETRY

Introduction

We have recently found that arterial oxygen saturation (SaO_2%), measured by pulsatile absorbance of light, can be readily determined in albino mice using a pulse oximeter (Figure XIII-1). An experiment was run to determine if this parameter could be used to measure ribavirin (AVS01) toxicity in mice.

Materials and Methods

Compounds: AVS01 was supplied by Biological Research Faculty and Facility, Inc. It was dissolved in sterile saline for use in this study.

Animals: Four-week-old female BALB/c mice were obtained from Simonsen Laboratories (Gilroy, CA). Following a 24 hr quarantine, the animals were used in this study. They were maintained on Wayne mouse chow and tap water *ad libitum*.

Pulse Oximeter: An Ohmeda Biox 3740 pulse oximeter (Ohmeda, Louisville, OH) was used. The Ohmeda Finger Probe clip, which sends a light beam through the mouse, was used.

Experiment Design: Ten mice were treated i.p. with 800 or 1200 mg/kg/day of ribavirin twice daily for 5 days. Pulse oximeter readings were taken each morning on days 1 through 4 of the experiment, and the animals were observed daily for occurrence of death. Pulse oximeter readings were expressed as SaO_2%, read directly from the instrument approximately 10 seconds after each mouse was placed in the finger probe clip. Hematocrit readings were taken from 5 mice in each group killed each day of the experiment.

Results and Discussion

This study is summarized in Figure XIII-2. SaO_2% began to decline by day 3 of treatment, continuing by day 4. By day 5, all the mice had died. Hematocrit readings (not shown in the figure) did not decline appreciably during this early phase of treatment. In our experience, anemia begins to appear with prolonged therapy.

By days 3 and 4, excessive hemorrhaging in the intestinal area was observed in sacrificed mice. This loss of blood, which would reduce the amount available for oxygen transport, would cause a significant lowering of the SaO_2%, which was observed in this study.

The pulse oximeter transmits light through the combination of blood and non-blood components of the finger, and, in our case, the entire mouse. The light transmitted through this pulsating vascular bed will be attenuated by the blood and non-blood components, but because the attenuation is pulsatile and assumed to result solely from the arterial component, the oximeter is calibrated to separate, literally by subtraction, the effects of the two components and can thus measure the SaO_2% of arterial blood in the mass. In the oximeter, two light-emitting diodes (660 nm and 940 nm) are mounted on one side of the vascular bed (in our case, the mouse), and a photodiode which converts light intensity into electrical current, is mounted on the opposite side. The diodes pulse at regular intervals and the photodiode measures the varying light intensities, which are changed to digital information processed by the algorithm in the oximeter. The Ohmeda Biox 3240 pulse oximeter (Ohmeda, Louisville, OH) used by us calculates SaO_2% as $K1(V)^2 + K2(V) + K3$, in which V is the change in the voltage in the red channel divided by the change in voltage in the infrared channel. K1, K2, and K3 are constants that are functions of the optical characteristics of hemoglobin as well as other variables.

These data indicate the pulse oximeter measurement of SaO_2% is an efficient means of measuring certain forms of toxicity in mice.

Conclusions

Ribavirin administered i.p. twice a day for 5 days in doses of 800 and 1200 mg/kg/day was lethally toxic to 4 week-old BALB/c mice. As the animals approached the time of death, which was attributed to excessive hemorrhaging in the gut, their arterial oxygen saturation (SaO_2%) declined appreciably.

Figure XIII-1. Use of the Ohmeda Biox 3740 Pulse Oximeter with Ear Probe for Monitoring SaO_2% in Mice.

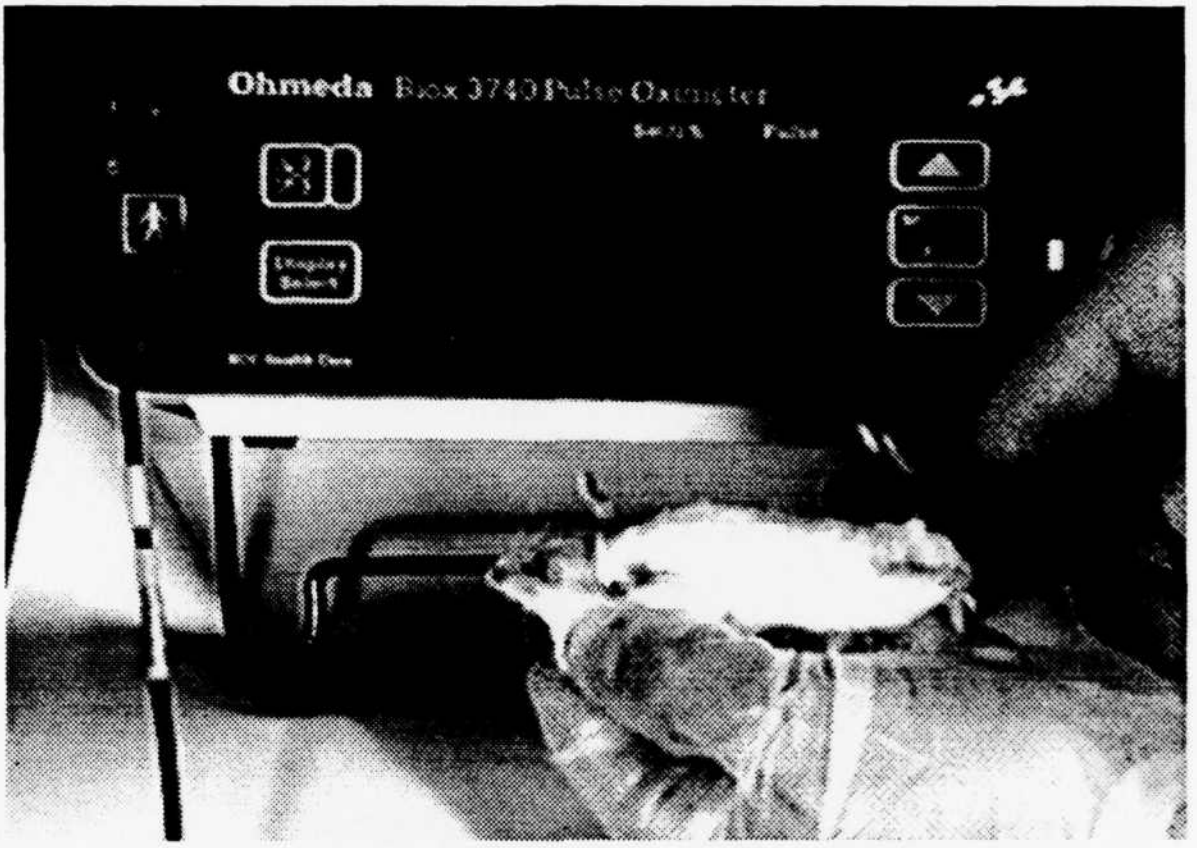
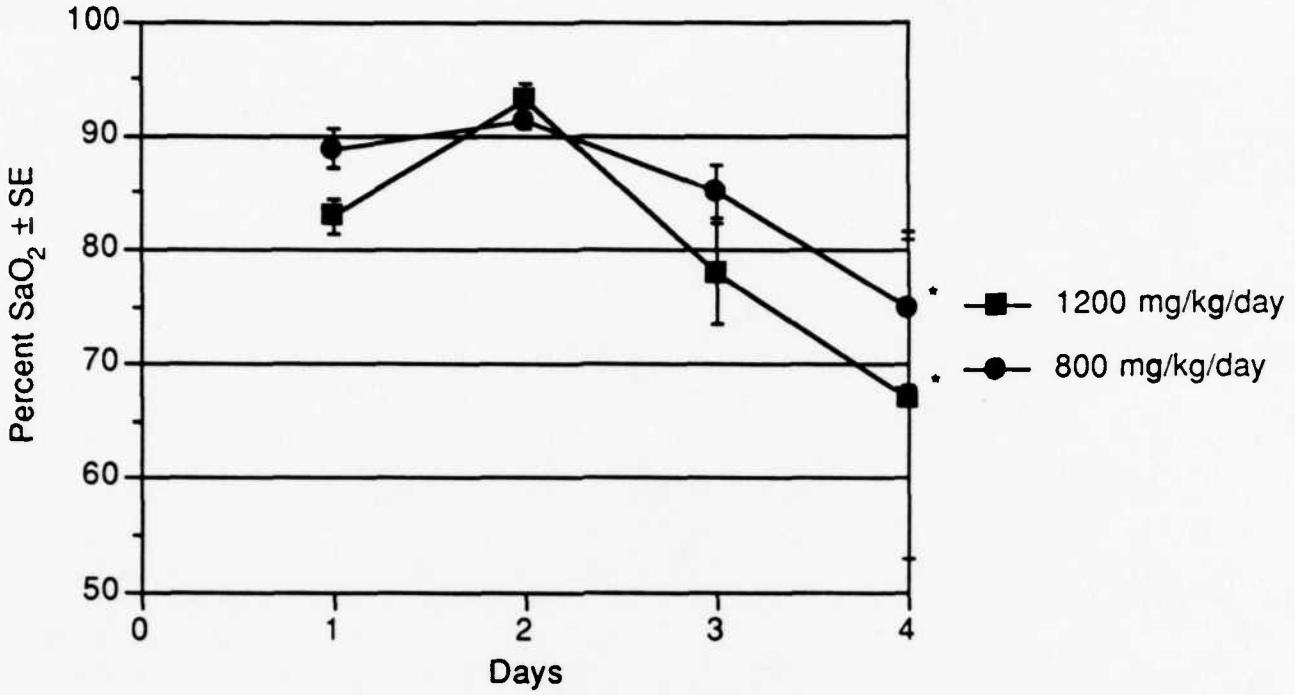


Figure XIII-2. Effect of Intraperitoneal AVS01[a] Treatment on SaO_2% in Uninfected Balb/c Mice.



[a]bid x 5.

*All animals died after these times.

Conclusions: High-dose AVS01 (ribavirin) treatment resulted in significant declines in SaO_2% prior to death of the animal. This decline correlated with the appearance of severe hemorrhaging in the intestinal tract of the animals.

XIV. OVERVIEW OF *IN VIVO* ANTI-PUNTA TORO VIRUS ACTIVITY OF AVS COMPOUNDS: SUMMARY OF FIVE YEARS' TESTING

Introduction

It is appropriate to summarize in tabular fashion all the *in vivo* work run to date against this virus. This table is shown in this section. All *in vivo* experiments, including both Adames and Balliet virus strains, combination studies, and special intravenous therapy studies are seen in Table XIV-1.

The following explains the legend for each column in the table:

AVS #: Number assigned to the compound by Biological Research Faculty & Facility, Inc.

Compound Name: Often an abbreviated name for the compound as provided to us. The short version of the name is used in order to fit it into the space provided.

Expt. #: The USU experiment number (PtA—). Every PTV *in vivo* experiment is numbered consecutively.

Test Date: The date the experiment was begun.

Treatment Schedule: The schedule used for the animal treatments, indicated in abbreviated form:

bid: Twice daily, usually 8 am and 4 pm
qd: Once daily
tid: Three times daily
single: Once only
qid: Four times daily
eod: Every other day
beg: Beginning, with the hrs indicated pre or post-virus inoculation; if no time is shown, virus was not given to the animals.

Route: Treatment route:
ip: intraperitoneal
sc: subcutaneous
po: oral gavage
ic: intracerebral
iv: intravenous.

Dose Range: Range of doses of the compound used, in mg/kg/day (unless actually shown as µg/kg/day or units/mouse). Doses usually varied by two-fold dilution, although some immunomodulators were used in one-half $\log_{10}$ increments.

Tox. @: The lowest dose (in mg/kg/day or, if indicated, as µg/kg/day) of the compound at which toxicity (death of one or more toxicity control animals) was seen. If a ">" sign is indicated, no toxicity was seen. "All lost weight" indicates the toxicity control mice all lost weight between the time therapy was initiated and 18 hr after treatment was terminated. "ON TEST" indicates the study was not sufficiently complete to indicate actual data at the time the table was prepared.

Results: Our overall impression of the antiviral efficacy seen:
+: Significant ($P<0.05$ or $P<0.01$) increase in survivors.
±: Significant effect on one or more parameters other than survivors (i.e., mean survival time increase; decrease in liver score, SGOT, SGPT, serum virus or liver virus) without a significant survivor increase.
-: No significant effects by any parameter.
TI: Therapeutic index (minimum toxic dose ÷ minimum antivirally effective dose).
?: Designation of a test in which the results were compromised by a poor control result.
ON TEST: Experiment still underway at the time the table was prepared.

MIC: Minimum inhibitory dose, in mg/kg/day or, if indicated in Dose Range column, in µg/kg/day or units/mouse.

Remarks:

EXPANDED: An experiment in which the infection parameters were expanded from survivors/total and mean survival time to include other parameters such as liver score, SGOT, SGPT, serum virus, liver virus, etc.

EXPANDED ALL: An experiment in which the infection parameters were expanded from a regular expanded study to also include many other tissues, such as spleen, lungs, mesenteric, brains, etc.

BALLIET: An experiment run using the Balliet strain of PTV. All other experiments using the Adames strain of PTV.

TI: Therapeutic index determination study.

MMF: Mode modification study (determination of effect of varying virus challenge inoculum concentration).

COMBINATION: An experiment in which a combination of two compounds were evaluated.

REPEAT: An experiment run to repeat a previous unacceptable experiment.

IFN: An experiment run to determine if the compound induced interferon in the animals, and the kinetics of that induction.

IMMUNOLOGY: Experiments in which immunological parameters other than IFN are studied with an immunomodulating compound.

TERMINATED: Experiment which was stopped early because of some error in treatment or infection.

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
1	Ribavirin	1	7-28-86	bid x 5, beg 4 hr pre	sc	9.4-75	75	+	9.4	EXPANDED
1	Ribavirin	6	10-16-86	bid x 9, beg 30 hr pre	sc	9.4-75	9.4	-	>75	BALLIET
1	Ribavirin	7	10-16-86	bid x 9, beg 30 hr pre	sc	9.4-75	9.4	-	>75	BALLIET
1	Ribavirin	8	10-23-86	bid x 7, beg 4 hr pre	sc	0.6-75	>75	TI 16	4.7	TI, MMF
1	Ribavirin	9	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	9.4	MMF
1	Ribavirin	10	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	9.4	MMF
1	Ribavirin	11	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	18.8	MMF
1	Ribavirin	20	1-16-87	bid x 5, beg 24 hr post	sc	37.5-150	150	+	37.5	EXPANDED
1	Ribavirin	21	1-16-87	bid x 5, beg 36 hr post	sc	37.5-150	150	+	37.5	EXPANDED
1	Ribavirin	28	1-22-87	single, beg 4 hr pre	sc	175-700	>700	?		
1	Ribavirin	29	1-22-87	single, beg 8 hr pre	sc	175-700	>700	?		
1	Ribavirin	30	1-22-87	single, beg 24 hr pre	sc	175-700	>700	?		
1	Ribavirin	31	1-22-87	single, beg 48 hr pre	sc	175-700	>700	?		
1	Ribavirin	32	1-22-87	single, beg 72 hr pre	sc	175-700	>700	?		
1	Ribavirin	33	1-22-87	single, beg 96 hr pre	sc	175-700	>700	?		
1	Ribavirin	43	2-5-87	bid x 5, beg 4 hr pre	po	3.2-100	>100	+	12.5	EXPANDED
1	Ribavirin	44	2-5-87	bid x 5, beg 4 hr post	po	3.2-100	>100	+	6.3	EXPANDED
1	Ribavirin	45	2-5-87	bid x 5, beg 24 hr post	po	3.2-100	>100	+	6.3	EXPANDED
1	Ribavirin	46	3-6-87	single, beg 4 hr post	sc	175-700	>700	+	175	
1	Ribavirin	47	3-6-87	single, beg 8 hr post	sc	175-700	>700	+	175	
1	Ribavirin	48	3-6-87	single, beg 24 hr post	sc	175-700	>700	+	175	
1	Ribavirin	49	3-6-87	single, beg 48 hr post	sc	175-700	>700	+	175	
1	Ribavirin	50	3-6-87	single, beg 72 hr post	sc	175-700	>700	±	350	
1	Ribavirin	51	3-6-87	single, beg 96 hr post	sc	175-700	>700	-	>700	
1	Ribavirin	162	10-16-87	bid x 5, beg 24 hr post	po	0.32-150	>150	+	32	COMBINATION
1	Ribavirin	193	11-13-87	bid x 5, beg 24 hr post	po	0.32-150	>150	+	10	COMBINATION
1	Ribavirin	427	7-7-88	bid x 5, beg 24 hr post	po	1-200	>200	+	32	COMBINATION
1	Ribavirin	537	11-22-88	single, beg 24 hr post	ic	43.75-350	43.8	-	>350	BALLIET
1	Ribavirin	577	01-05-89	bid x 5, beg 24 hr post	po	1-300	>300	+	1	COMBINATION
1	Ribavirin	584	01-11-89	single, beg 4 hr pre	iv	62.5-500	>4500	±	500	BALLIET
1	Ribavirin	647	03-16-89	bid x 3, beg 24 hr post	po	3.13-1200	>1200	+	12.5	COMBINATION
1	Ribavirin	669	04-19-89	bid x 5, beg 24 hr post	sc	3.2-1000	1000	+	3.2	EXPANDED ALL
1	Ribavirin	687	05-17-89	bid x 5, beg 24 hr post	po	6.4-2000	2000	+	6.4	EXPANDED ALL
1	Ribavirin	690	05-25-89	qd x5, varying times	sc	140	>140	+	140	
1	Ribavirin	693	06-02-89	bid x 5, varying times	sc	140	>140	+	140	
1	Ribavirin	696	06-08-89	bid x 5, varying times	po	325	>325	+	48 post	
1	Ribavirin	701	07-14-89	qd x 5, varying times	po	325	>325	+	72 post	
1	Ribavirin	704	07-14-89	bid x 1-5, beg 24 hr post	po	325	~325	+	325	
1	Ribavirin	705	07-14-89	single, beg 24 hr post	po	325	~325	+	325	
1	Ribavirin	711	07-14-89	bid x 5, beg 4 hr post	sc	16	>16	?	?	BALLIET
1	Ribavirin	712	07-20-89	bid x 1-5, beg 24 hr post	sc	140	>140	+	140	
1	Ribavirin	713	07-20-89	single, beg 24 hr post	sc	140	>140	+	140	
1	Ribavirin	719	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	720	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	721	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	722	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	723	07-28-89	bid x 5, beg 24 hr post	po	7.5-750	>750	+	75	MMF
1	Ribavirin	736	08-10-89	bid x 1-5, beg 24 hr post	po	81	>81	+	81	

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
1	Ribavirin	737	08-10-89	single, beg 24 hr post	po	81	>81	+	81	
1	Ribavirin	761	09-15-89	bid x 5, beg 4 hr pre	ip	75-600	600	±	300	BALLIET
1	Ribavirin	765	09-21-89	bid x 1-5, beg 24 hr post	po	20	>20	+	20	
1	Ribavirin	766	09-21-89	single, beg 24 hr post	po	20	>20	+	20	
1	Ribavirin	771	09-27-89	single, beg 24 hr post	po	41	>41	+	41	EXPANDED ALL
1	Ribavirin	774	10-06-89	bid x 3, beg 24 hr post	po	6 25-1250	1250	+	25	COMBINATION
1	Ribavirin	788	11-03-89	bid x 5, beg 4 hr post	sc	16	>16	ON TEST	ON TEST	EXPANDED ALL
1	Ribavirin	813	02-22-90	bid x 3, beg 24 hr post	po	1 60-2000	2000	+	1 6	COMBINATION
1	Ribavirin	844	06-21-90	bid x 3, beg 24 hr post	po	-146060	1200	±	10	COMBINATION
2	Ribavirin triacetate	106	8-14-87	bid x 5, beg 4 hr pre	sc	25-200	>200	+	25	
2	Ribavirin triacetate	112	8-21-87	bid x 5, beg 4 hr pre	sc	15 6-500	>500	TI 16	62 5	EXPANDED
2	Ribavirin triacetate	113	8-21-87	single, beg 4 hr post	sc	62.5-1000	>1000	+	62.5	
2	Ribavirin triacetate	114	8-21-87	single, beg 24 hr post	sc	62.5-1000	>1000	+	62.5	
2	Ribavirin triacetate	115	8-21-87	single, beg 48 hr post	sc	62.5-1000	>1000	+	62.5	
2	Ribavirin triacetate	116	8-21-87	single, beg 72 hr post	sc	62.5-1000	>1000	-	>1000	
2	Ribavirin triacetate	117	8-21-87	single, beg 96 hr post	sc	62.5-1000	>1000	-	>1000	
2	Ribavirin triacetate	134	9-18-87	bid x 5, beg 24 hr pre	po	9 4-600	600	TI 8	37 5	EXPANDED
2	Ribavirin triacetate	167	10-22-87	bid x 5, beg 4 hr pre	ip	125-1000	1000	+	250	BALLIET
2	Ribavirin triacetate	177	10-30-87	qd x 5, beg 4 hr pre	sc	62.5-500	>500	?		
2	Ribavirin triacetate	178	10-30-87	bid x 5, beg 4 hr pre	sc	62.5-500	>250	+	31.3	MMF
2	Ribavirin triacetate	179	10-30-87	bid x 5, beg 4 hr pre	sc	62.5-500	>250	+	62.5	MMF
2	Ribavirin triacetate	180	10-30-87	qd x 5, beg 4 hr pre	sc	62.5-500	>250	+	62 5	MMF
2	Ribavirin triacetate	181	10-30-87	qd x 5, beg 4 hr pre	sc	62 5-500	>250	+	62.5	MMF
2	Ribavirin triacetate	185	11-6-87	qd x 5, beg 4 hr pre	sc	31 3-1000	>1000	TI 16	62.5	
2	Ribavirin triacetate	339	4-15-88	single, beg 24 hr post	po	62.5-500	>500	+	62 5	EXPANDED
2	Ribavirin triacetate	340	4-15-88	single, beg 48 hr post	po	62.5-500	>500	+	250	EXPANDED
2	Ribavirin triacetate	377	5-20-88	bid x 5, beg 24 hr post	po	31.3-500	>500	+	31.3	EXPANDED
2	Ribavirin triacetate	378	5-20-88	bid x 5, beg 48 hr post	po	31.3-500	>500	+	31.3	EXPANDED
2	Ribavirin triacetate	671	04-19-89	bid x 5, beg 24 hr post	sc	9 6-3000	3000	+	9.6	EXPANDED ALL
2	Ribavirin triacetate	689	05-17-89	bid x 5, beg 24 hr post	po	12 8-4000	4000	+	12 8	EXPANDED ALL
2	Ribavirin triacetate	692	05-25-89	qd x 5, varying times	sc	425	>425	+	425	
2	Ribavirin triacetate	695	06-02-89	bid x 5, varying times	sc	425	>425	+	425	
2	Ribavirin triacetate	698	06-08-89	bid x 5, varying times	po	650	>563	+	96 post	
2	Ribavirin triacetate	702	07-14-89	qd x 5, varying times	po	563	>563	+	48 post	
2	Ribavirin triacetate	706	07-14-89	bid x 1-5, beg 24 hr post	po	563	>563	+	563	
2	Ribavirin triacetate	707	07-14-89	single, beg 24 hr post	po	563	>563	+	563	
2	Ribavirin triacetate	714	07-20-89	bid x 1-5, beg 24 hr post	sc	425	>425	+	425	
2	Ribavirin triacetate	715	07-20-89	single, beg 24 hr post	sc	425	>425	+	425	
2	Ribavirin triacetate	724	07-28-89	bid x 5, beg 24 hr post	po	11.3-1126	>1126	+	112.6	MMF
2	Ribavirin triacetate	725	07-28-89	bid x 5, beg 24 hr post	po	11.3-1126	>1126	+	112.6	MMF
2	Ribavirin triacetate	726	07-28-89	bid x 5, beg 24 hr post	po	11.3-1126	>1126	+	112.6	MMF
2	Ribavirin triacetate	727	07-28-89	bid x 5, beg 24 hr post	po	11 3-1126	>1126	+	112.6	MMF
2	Ribavirin triacetate	728	07-28-89	bid x 5, beg 24 hr post	po	11 3-1126	>1126	+	11.3	MMF
2	Ribavirin triacetate	738	08-10-89	bid x 1-5, beg 24 hr post	po	141	>141	+	141	
2	Ribavirin triacetate	739	08-10-89	single, beg 24 hr post	po	141	>141	+	141	
2	Ribavirin triacetate	762	09-15-89	bid x 5, beg 4 hr pre	ip	225-1800	900	-	>1800	BALLIET
2	Ribavirin triacetate	767	09-21-89	bid x 1 5, beg 24 hr post	po	35	>35	+	35	
2	Ribavirin triacetate	768	09-21-89	single, beg 24 hr post	po	35	>35	+	35	

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2	Ribavirin triacetate	772	09-27-89	single, beg 24 hr post	po	71	>71	+	71	EXPANDED ALL
52	Thioformycin B	2	10-10-86	bid x 5, beg 4 hr pre	sc	62.5-250	>250	-	>250	
52	Thioformycin B	22	1-22-87	single, beg 4 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	23	1-22-87	single, beg 8 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	24	1-22-87	single, beg 24 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	153	10-9-87	tid x 5, beg 4 hr pre	sc	62.5-500	>500	+	250	
52	Thioformycin B	231A	12-18-87	qid x 5, beg 4 hr pre	sc	25-400	>400	±	50	
52	Thioformycin B	342	4-22-88	tid x 5, beg 4 hr pre	po	50-400	>400	+	50	EXPANDED
65	Formycin B	52	3-12-87	bid x 5, beg 4 hr pre	sc	62.5-250	>250	-	>250	
65	Formycin B	551	12-01-88	tid x 5, beg 4 hr pre	sc	31.3-500	>500	+	125	
65	Formycin B	560	12-08-88	single, beg 4 hr pre	sc	31.3-500	>500	-	>500	
65	Formycin B	561	12-08-88	single, beg 24 hr post	sc	31.3-500	>500	+	62.5	
65	Formycin B	596	01-19-89	single, beg 24 hr post	sc	100-800	>800	-	>800	
65	Formycin B	597	01-19-89	single, beg 24 hr post	ip	100-800	>800	+	200	
65	Formycin B	806		bid x 5, beg 4 hr pre	sc	62.5-500	>500	+	62.5	EXPANDED
65	Formycin B	811	02-08-90	tid x 5, beg 4 hr pre	ip	62.5-500	>500	±	125	EXPANDED
65	Formycin B	818	03-01-90	tid x 5, beg 4 hr pre	ip	125-1000	1000	-	>1000	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	3	10-10-86	bid x 5, beg 4 hr pre	sc	25-100	100	+	25	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	12	11-14-86	bid x 5, beg 4 hr pre	sc	6.25-50	>50	TI 2	6.25	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	18	12-3-86	bid x 5, beg 24.4 hr pre	sc	9.4-75	>75	-	>75	BALLIET
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	25	1-22-87	single, beg 4 hr post	sc	175-700	700	?		
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	26	1-22-87	single, beg 8 hr post	sc	175-700	700	?		
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	27	1-22-87	single, beg 24 hr post	sc	175-700	700	?		
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	95	7-30-87	qd x 5, beg 4 hr pre	sc	25-200	200	?		
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	102	8-7-87	bid x 5, beg 4 hr pre	po	25-200	>200	±	>200	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	107	8-14-87	bid x 5, beg 24 hr post	sc	18.8-150	>150	-	18.8	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	108	8-14-87	bid x 5, beg 36 hr post	sc	18.8-150	>150	-	37.5	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	109	8-14-87	bid x 5, beg 48 hr post	sc	18.8-150	>150	-	>150	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	133	9-11-87	qd x 5, beg 4 hr pre	sc	25-200	200	+	50	REPEAT #95
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	154	10-9-87	single, beg 4 hr post	sc	87.5-700	>700	±	350	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	155	10-9-87	single, beg 24 hr post	sc	87.5-700	>700	±	175	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	156	10-9-87	single, beg 48 hr post	sc	87.5-700	>700	+	87.5	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	157	10-9-87	single, beg 72 hr post	sc	87.5-700	>700	-	>700	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	158	10-9-87	single, beg 96 hr post	sc	87.5-700	>700	-	>700	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	187	11-6-87	bid x 5, beg 4 hr pre	ip	6.25-200	200	-	200	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	188	11-6-87	tid x 5, beg 4 hr pre	sc	6.25-200	200	+	6.25	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	336	4-15-88	single, beg 4 hr post	po	87.5-700	>700	±	175	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	337	4-15-88	single, beg 24 hr post	po	87.5-700	>700	+	87.5	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	338	4-15-88	single, beg 48 hr post	po	87.5-700	>700	+	87.5	EXPANDED
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	374	05-20-88	single, beg 4 hr post	po	87.5-700	>700	±	350	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	375	05-20-88	single, beg 24 hr post	po	87.5-700	>700	±	700	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	376	05-20-88	single, beg 48 hr post	po	87.5-700	>700	±	175	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	403	6-17-88	single, beg 60 hr post	po	43.8-700	>700	-	>700	
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	534	11-22-88	single, beg 24 hr post	ip	62.5-500	>500	-	>500	BALLIET
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	799	01-04-90	tid x 4, beg 4 hr pre	ip	125-1000	250			
79	9-ß-D-ribofuranosylpurine-6-thiocarboxamide	819	03-01-90	tid x 5, beg 4 hr pre	ip	7.8-62.5	>62.5	+	15.6	EXPANDED
111	Tiazofurin	53	3-12-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	+	62.5	
111	Tiazofurin	68	3-26-87	bid x 5, beg 4 hr pre	sc	31.3-250	>500	+	31.3	EXPANDED

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
111	Tiazofurin	110	8-14-87	bid x 5, beg 4 hr pre	sc	15.7-2000	2000	TI=8-16	125	TI
111	Tiazofurin	135	9-18-87	single, beg 4 hr post	sc	125-1000	250	+	250	
111	Tiazofurin	136	9-18-87	single, beg 24 hr post	sc	125-1000	250	+	1000	
111	Tiazofurin	137	9-18-87	single, beg 48 hr post	sc	125-1000	250	+	250	
111	Tiazofurin	138	9-18-87	single, beg 72 hr post	sc	125-1000	250	-	>1000	
111	Tiazofurin	139	9-18-87	single, beg 96 hr post	sc	125-1000	250	±	1000	
111	Tiazofurin	182	11-5-87	bid x 5, beg 24 hr pre	sc	62.5-500	>500	-	>500	BALLIET
111	Tiazofurin	365	5-6-88	bid x 5, beg 4 hr pre	po	93.8-750	>750	+	93.8	EXPANDED
111	Tiazofurin	832	04-19-90	bid x 5, beg 4 hr pre	sc	62.5-1000	>1000	+	125	EXPANDED
147	Enviroxime	15	11-19-86	bid x 5, beg 4 hr pre	sc	25-100	>100	-	>100	
147	Enviroxime	34	1-29-87	single, beg 4 hr post	sc	250-1000	>1000	+	1000	
147	Enviroxime	35	1-29-87	single, beg 12 hr post	sc	250-1000	>1000	±	>1000	
147	Enviroxime	36	1-29-87	single, beg 24 hr post	sc	250-1000	>1000	-	>1000	
147	Enviroxime	96	7-30-87	qd x 5, beg 4 hr pre	sc	62.5-500	>500	-	>500	
147	Enviroxime	371	5-13-88	single, beg 4 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	372	5-13-88	single, beg 24 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	373	5-13-88	single, beg 48 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	522	11-02-88	single, beg 24 hr pre	po	150-1200	1200	-	>1200	EXPANDED
147	Enviroxime	523	11-03-88	single, beg 4 hr post	po	150-1200	1200	±	300	EXPANDED
147	Enviroxime	524	11-03-88	single, beg 24 hr post	po	150-1200	1200	-	>1200	EXPANDED
147	Enviroxime	817	03-01-90	tid x 5, beg 4 hr pre	sc	75-500	>500	±	75	EXPANDED
147	Enviroxime	820	03-08-90	bid x 5, beg 4 hr pre	sc	75-500	>500	±	125	EXPANDED
167	Glycerrhetic Acid	54	3-12-87	bid x 5, beg 4 hr pre	sc	18.8-75	>75	-	>75	
167	Glycerrhetic Acid	87	4-24-87	bid x 5, beg 4 hr pre	sc	62.5-500	>500	-	500	REPEAT
167	Glycerrhetic Acid	304	3-3-88	tid x 5, beg 24 hr pre	ip	75-600	300	-	>600	
206	Ribamidine	4	10-10-86	bid x 5, beg 4 hr pre	sc	125-500	>500	+	125	
206	Ribamidine	13	11-14-86	bid x 5, beg 4 hr pre	sc	31.3-250	>250	+	31.3	
206	Ribamidine	71	4-3-87	bid x 5, beg 4 hr pre	sc	3.9-1000	>1000	TI >32	31.3	TI
206	Ribamidine	78	4-10-87	bid x 5, beg 24 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	79	4-10-87	bid x 5, beg 36 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	80	4-10-87	bid x 5, beg 48 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	81	4-10-87	bid x 5, beg 72 hr post	sc	62.5-500	>500	+	125	EXPANDED
206	Ribamidine	86	4-23-87	bid x 5, beg 24 hr pre	sc	125-500	>500	-	125	BALLIET
206	Ribamidine	92	7-28-87	bid x 5, beg 4 hr pre	po	7.8-1000	>1000	TI≥564	31.3	TI
206	Ribamidine	169	10-23-87	single, beg 4 hr post	sc	15.7-1000	>1000	+	62.5	
206	Ribamidine	170	10-23-87	single, beg 24 hr post	sc	15.7-1000	>1000	+	500	
206	Ribamidine	171	10-23-87	single, beg 48 hr post	sc	15.7-1000	>1000	+	250	
206	Ribamidine	172	10-23-87	single, beg 72 hr post	sc	15.7-1000	>1000	-	>1000	
206	Ribamidine	173	10-23-87	single, beg 96 hr post	sc	15.7-1000	>1000	-	>1000	
206	Ribamidine	233	12-18-87	bid x 5, beg 4 hr pre	sc	7.8-2000	2000	+		
206	Ribamidine	234	12-18-87	bid x 5, beg 4 hr pre	po	7.8-2000	2000	+		
206	Ribamidine	287	2-19-88	bid x 5, beg 24 hr post	po	2.4-75	>75	+	2.4	COMBINATION
206	Ribamidine	363	5-6-88	bid x 5, beg 24 hr pre	ip	75-600	>600	±	600	BALLIET
206	Ribamidine	382	5-27-88	bid x 5, beg 18 hr post	po	2.4-75	>75	+	18.8	COMBINATION
206	Ribamidine	447	8-5-88	bid x 3, beg 24 hr post	po	1000	>1000	+	1000	
206	Ribamidine	535	11-22-88	single, beg 24 hr post	ip	250-2000	>2000	±	2000	BALLIET
206	Ribamidine	536	11-22-88	single, beg 24 hr post	ic	62.5-1000	500	-	>1000	BALLIET
206	Ribamidine	670	04-19-89	bid x 5, beg 24 hr post	sc	9.6-3000	3000	+	30	EXPANDED ALL

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
206	Ribamidine	688	05-17-89	bid x 5, beg 24 hr post	po	12.8-4000	4000	+	12.8	EXPANDED ALL
206	Ribamidine	691	05-25-89	qd x 5, varying times	sc	425	>425	+	425	
206	Ribamidine	694	06-02-89	bid x 5, varying times	sc	425	>425	+	425	
206	Ribamidine	697	06-08-89	bid x 5, varying times	po	650	>650	+	48 post	
206	Ribamidine	703	07-14-89	qd x 5, varying times	po	650	>650	+	48 post	
206	Ribamidine	708	07-14-89	bid x 1-5, beg 24 hr post	po	650	>650	+	650	
206	Ribamidine	709	07-14-89	single, beg 24 hr post	po	650	>650	+	650	
206	Ribamidine	716	07-20-89	bid x 1-5, beg 24 hr post	sc	425	>425	+	425	
206	Ribamidine	717	07-20-89	single, beg 24 hr post	sc	425	>425	+	425	
206	Ribamidine	729	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	13	MMF
206	Ribamidine	730	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	731	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	732	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	130	MMF
206	Ribamidine	733	07-28-89	bid x 5, beg 24 hr post	po	13-1300	>1300	+	41.1	MMF
206	Ribamidine	740	08-10-89	bid x 1-5, beg 24 hr post	po	163	>163	+	163	
206	Ribamidine	741	08-10-89	single, beg 24 hr post	po	163	>163	+	163	
206	Ribamidine	763	09-15-89	bid x 5, beg 4 hr pre	ip	225-1800	1800	-	>1800	BALLIET
206	Ribamidine	769	09-21-89	bid x 1-5, beg 24 hr post	po	41	>41	+	41	
206	Ribamidine	770	09-21-89	single, beg 24 hr post	po	41	>41	+	41	
206	Ribamidine	773	09-27-89	single, beg 24 hr post	po	82	>82	+	82	EXPANDED ALL
212	Suramin	16	11-19-86	bid x 5, beg 4 hr pre	sc	18.8-75	>25	-	>75	
212	Suramin	37	1-29-87	single, beg 4 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	38	1-29-87	single, beg 12 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	39	1-29-87	single, beg 24 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	103	8-7-87	bid x 5, beg 4 hr pre	po	75-200	>200	-	>200	EXPANDED
212	Suramin	159	10-9-87	tid x 5, beg 4 hr pre	sc	18.8-150	>150	-	>150	
215	3-Deazaguanosine	497	10-13-88	qd x 5, beg 4 hr pre	sc	18.8-300	150	±	37.5	
215	3-Deazaguanosine	557	12-08-88	tid x 5, beg 4 hr pre	sc	12.5-100	>100	-	>100	
215	3-Deazaguanosine	558	12-08-88	bid x 5, beg 4 hr pre	sc	12.5-100	>100	+	50	
215	3-Deazaguanosine	559	12-08-88	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	25	
215	3-Deazaguanosine	591	01-19-89	tid x 5, beg 4 hr pre	ip	12.5-100	>100	+	12.5	EXPANDED
215	3-Deazaguanosine	592	01-19-89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	25	EXPANDED
215	3-Deazaguanosine	646	03-09-89	bid x 5, beg 24 hr pre	ip	3.13-50	>50	-	>50	BALLIET
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	55	3-12-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	-	>250	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	88	4-24-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	-	31.3	EXPANDED
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	302	3-3-88	qd x 5, beg 24 hr pre	sc	62.5-500	>500	-	>500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	366	5-6-88	bid x 5, beg 4 hr pre	sc	2000	2000	-	>2000	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	437	7-20-88	single, 24 hr pre	ip	187.5-1500	>1500	±	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	438	7-21-88	single, 4 hr pre	ip	187.5-1500	>1500	-	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	439	7-21-88	single, 24 hr post	ip	187.5-1500	>1500	-	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	440	7-21-88	tid x 5, beg 4 hr pre	sc	62.5-500	>500	-	>500	
233	Formycin	17	11-19-86	bid x 5, beg 4 hr pre	sc	100-400	>400	-	>400	
233	Formycin	40	1-29-87	single, beg 12 hr post	sc	450-1800	900	±	450	
233	Formycin	41	1-29-87	single, beg 12 hr post	sc	450-1800	900	±	450	
253	Selenazofurin	5	10-10-86	bid x 5, beg 4 hr pre	sc	80-320	160	+	80	
253	Selenazofurin	14	11-14-86	bid x 5, beg 4 hr pre	sc	20-160	>160	+	80	
253	Selenazofurin	19	12-3-86	bid x 5, beg 24,4 hr pre	sc	18.8-150	>150	+	>150	BALLIET
253	Selenazofurin	97	7-30-87	qd x 5, beg 4 hr pre	sc	40-320	320	±	40	REPEAT

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
253	Selenazofurin	104	8-7-87	bid x 5, beg 4 hr pre	po	40-320	320	+	40	EXPANDED
253	Selenazofurin	538	11-22-88	single, beg 4 hr post	ip	93.75-750	>750	±	93.8	BALLIET
253	Selenazofurin	800	01-04-90	qd x 4, beg 4 hr pre	ip	125-1000	>1000	+		EXPANDED
253	Selenazofurin	801	01-04-90	bid x 5, beg 4 hr pre	ip	125-1000	1000	+		EXPANDED
257	Tiazofurin 5'-MP	445	7-21-88	bid x 5, beg 4 hr pre	ip	25-400	>400	+	400	
257	Tiazofurin 5'-MP	449	9-2-88	bid x 5, beg 4 hr pre	ip	50-400	>400	+	200	EXPANDED
272	3-Deazaguanine	186	11-6-87	bid x 5, beg 4 hr pre	sc	25-200	100	-	>200	
272	3-Deazaguanine	232	12-18-87	qd x 5, beg 4 hr pre	sc	25-200	>200	+	25	
272	3-Deazaguanine	280	2-11-86	bid x 5, beg 24 hr pre	ip	12.5-100	>100	?		
272	3-Deazaguanine	317	3-18-88	bid x 5, beg 24 hr pre	ip	12.5-100	100	-	>12.5	
272	3-Deazaguanine	343	4-22-88	qd x 5, beg	ip	25-200	200	-	>200	BALLIET
272	3-Deazaguanine	370	5-13-88	qd x 5, beg 4 hr pre	po	18.8-300	>300	+	18.8	EXPANDED
272	3-Deazaguanine	498	10-13-88	qd x 5, beg 4 hr pre	sc	18.8-300	>300	-	>300	EXPANDED
272	3-Deazaguanine	539	11-22-88	single, beg 4 hr post	ip	93.75-750	>750	-	>750	BALLIET
272	3-Deazaguanine	802	01-12-90	bid x 5, beg 4 hr pre	sc	62.5-500	500	+	62.5	EXPANDED
347	Phyllanthoside	829	04-12-90	bid x 4, beg 4 hr pre	sc	15-120	120	±	30	EXPANDED
360	7-Deoxynarciclasin	42	1-29-87	bid x 5, beg 4 hr pre	sc	62.5-500	>500	±	250	
361	Pancratistatin	417	6-24-88	qd x 7, beg 24 hr pre	sc	.5-4	>4	-	>4	
1018	Phenyleneamine	791	11-16-89	single, beg 4 hr post	po	1.56-12.5	>12.5	±	12.5	EXPANDED
1018	Phenyleneamine	792	11-16-89	3 shots in 9 days, beg 24 hr post	po	1.56-12.5	>12.5	+	1.56	EXPANDED
1018	Phenyleneamine	830	04-12-90	single, beg 24 hr post	po	3.13-25	>25	+	6.25	EXPANDED
1018	Phenyleneamine	831	04-12-90	single, beg 36 hr post	po	3.13-25	>25	+	6.25	EXPANDED
1018	Phenyleneamine	838	05-31-90	4 hr pre, day 4	po	3.13-25	>25	±	3.13	BALLIET
1212	Uridine 2',3'-dialdehyde	550	12-01-88	bid x 5, beg 4 hr pre	sc	25-400	>400	-	>400	INITIAL
1212	Uridine 2',3'-dialdehyde	562	12-08-88	single, beg 4 hr pre	sc	25-400	>400	±	25	
1212	Uridine 2',3'-dialdehyde	563	12-08-88	single, beg 24 hr post	sc	25-400	>400	+	25	
1212	Uridine 2',3'-dialdehyde	598	01-19-89	single, beg 24 hr post	sc	12.5-100	>100	-	>100	
1212	Uridine 2',3'-dialdehyde	599	01-19-89	single, beg 24 hr post	ip	12.5-100	>100	+	100	
1212	Uridine 2',3'-dialdehyde	661	04-06-89	single, beg 24 hr post	ip	100-800	800	±	100	EXPANDED
1754	MVE-2	58	3-19-87	single, beg 24 hr pre	ip	6.25-50	>50	+	12.5	
1754	MVE-2	89	4-23-87	single, beg 24 hr pre	ip	6.25-50	>50	+	6.25	EXPANDED
1754	MVE-2	98	7-30-87	single, beg 4 hr pre	ip	6.25-100	25	+	6.3	
1754	MVE-2	99	7-30-87	single, beg 4 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	100	7-30-87	single, beg 24 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	101	7-30-87	single, beg 48 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	151	10-1-87	single, beg 24 hr pre	po	6.25-200	>200	-	>200	EXPANDED
1754	MVE-2	161	10-8-87	single, beg 4 hr pre	ip	12.5-100	100	±	50	BALLIET
1754	MVE-2	238	01-08-88	qd x 3, beg 4 hr pre	ip	3.13-50	50	+	6.25	
1754	MVE-2	240	01-08-88	single, beg 72 hr post	ip	6.25-100	>100	-	>100	
1754	MVE-2	241	01-08-88	single, beg 96 hr post	ip	6.25-100	>100	-	>100	
1754	MVE-2	249	01-15-88	single, beg 4 hr pre	sc	6.25-100	12.5	+	6.25	
1754	MVE-2	252	1-14-88	single	ip	6.25-100	>100	±	12.5	IFN
1754	MVE-2	311	3-11-88	bid x 5, beg 4 hr pre	ip	6.25-50	>50	+	6.25	
1754	MVE-2	431	7-7-88	single, beg 24 hr post	ip	0.05, 0.5, 5	>5	+	5	IFN, EXPANDED
1754	MVE-2	603	01-26-89	bid x 5, beg 24 hr post	ip	1.56-50	>50	+	12.5	EXPANDED
1754	MVE-2	624	02-24-89	single, beg 24 hr post	ip	0.75-25	>25	±	12.5	MMF
1754	MVE-2	625	02-24-89	single, beg 24 hr post	ip	0.75-25	>25	+	12.5	MMF
1754	MVE-2	626	02-24-89	single, beg 24 hr post	ip	0.75-25	>25	+	12.5	MMF

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
1754	MVE-2	627	02-24-89	single, beg 24 hr post	ip	0.75-25	>25	+	12.5	MMF
1757	Isoprinosine	76	4-10-87	bid x 5, beg 24 hr post	po	250-1000	>1000	-	>1000	
1761	Poly IC-LC	307	3-3-88	qd x 8, beg 24 hr pre	ip	0.0195-5	5	+	0.0195	
1761	Poly IC-LC	324	3-24-88	qd x 8, beg 24 hr pre	sc	0.031-1	>1	+	0.031	EXPANDED
1761	Poly IC-LC	325	3-24-88	qd x 8, beg 24 hr pre	po	0.031-1	>1	-	1	EXPANDED
1761	Poly IC-LC	326	3-25-88	single, beg 4 hr pre	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC-LC	327	3-25-88	single, beg 4 hr post	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC-LC	328	3-25-88	single, beg 24 hr post	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC-LC	329	3-25-88	single, beg 48 hr post	ip	0.31-2.5	>2.5	+	0.625	
1761	Poly IC-LC	330	3-25-88	single, beg 72 hr post	ip	0.31-2.5	>2.5	-	>2.5	
1761	Poly IC-LC	331	3-25-88	single, beg 96 hr post	ip	0.31-2.5	>2.5	-	>2.5	
1761	Poly IC-LC	361	4-29-88	qd x 5, beg 4 hr pre	sc	0.0625-0.5	>0.5	-	>0.5	BALLIET
1761	Poly IC-LC	672	04-27-89	3 in 7 days, beg 4 hr post	ip	0.125-1	>1	+	0.125	EXPANDED
1761	Poly IC-LC	734	08-04-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.0032	EXPANDED
1761	Poly IC-LC	742	08-10-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
1761	Poly IC-LC	745		eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
1761	Poly IC-LC	749	08-24-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
1761	Poly IC-LC	814	02-22-90	eod x 3, beg 24 hr post	ip	0.32	>0.32	+	0.32	COMBINATION
1761	Poly IC-LC	821	03-08-90	eod x 3, beg 24 hr post	ip	0.001-0.01	>0.01	+	0.001	COMBINATION
1767	AM-3	72	4-3-87	bid x 5, beg 4 hr pre	sc	112.5-450	>450	+	112.5	
1767	AM-3	73	4-3-87	bid x 5, beg 4 hr pre	po	112.5-450	>450	-	>450	
1767	AM-3	111	8-14-87	bid x 5, beg 4 hr pre	sc	62.5-2000	2000	+	62.5	EXPANDED
1767	AM-3	168	10-22-87	bid x 5, beg 24 hr pre	ip	62.5-500	500	-	>500	BALLIET
1767	AM-3	243	01-15-88	single, beg 4 hr pre	sc	25-400	>400	+	50	
1767	AM-3	244	01-15-88	single, beg 4 hr post	sc	25-400	>400	+	25	
1767	AM-3	245	01-15-88	single, beg 24 hr post	sc	25-400	>400	+	25	
1767	AM-3	246	01-15-88	single, beg 48 hr post	sc	25-400	>400	+	25	
1767	AM-3	247	01-15-88	single, beg 72 hr post	sc	25-400	>400	-	>400	
1767	AM-3	248	01-15-88	single, beg 96 hr post	sc	25-400	>400	-	>400	
1767	AM-3	251	1-14-88	single	sc	25-400	>400	-	>400	IFN
1767	AM-3	259	1-29-88	qd x 5, beg 4 hr pre	sc	31.3-250	>250	+	62.5	
1767	AM-3	260	1-29-88	single, beg 4 hr pre	sc	15.6-1000	1000	+	15.6	
1767	AM-3	261	1-29-88	single, beg 4 hr post	sc	15.6-1000	1000	+	62.5	
1767	AM-3	262	1-29-88	single, beg 24 hr post	sc	15.6-1000	1000	±	62.5	
1767	AM-3	263	1-29-88	single, beg 48 hr post	sc	15.6-1000	1000	+	15.6	
1767	AM-3	264	1-29-88	single, beg 72 hr post	sc	15.6-1000	1000	±	500	
1767	AM-3	265	1-29-88	single, beg 96 hr post	sc	15.6-1000	1000	±	15.6	
1767	AM-3	267	1-29-88	single	sc	31.3-250	>250	-	>250	IFN
1767	AM-3	308	3-11-88	bid x 5, beg 4 hr pre	ip	15.7-250	>250	+	15.7	
1767	AM-3	386	5-27-88	single, beg 48 hr post	sc	5, 16, 50	>50	-	50	COMBINATION
1767	AM-3	540	11-22-88	single, beg 4 hr post	sc	62.5-500	>500	-	>500	BALLIET
1777	Streptonigrin	77	4-10-87	qd x 5, beg 4 hr pre	sc	0.125-1	0.5	-	>1	
1777	Streptonigrin	566	12-14-88	single, beg 24 hr pre	ip	0.31-5	1.25	-	>5	
1777	Streptonigrin	567	12-14-88	single, beg 4 hr post	ip	0.31-5	1.25	-	>5	
1777	Streptonigrin	568	12-14-88	single, beg 24 hr post	ip	0.31-5	1.25	-	>5	
1777	Streptonigrin	569	12-15-88	bid x 5, beg 4 hr pre	ip	0.125-1	0.5	-	>1	
1777	Streptonigrin	570	12-15-88	tid x 5, beg 4 hr pre	ip	0.125-1	0.5	-	>1	
1778	Mannozym	74	4-3-87	single, beg 4 hr pre	sc	12.5 50	50	+	25	

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
1778	Mannozym	75	4-3-87	bid x 5, beg 4 hr pre	sc	3.1-50	>50	+	3.13	
1778	Mannozym	93	7-28-87	bid x 5, beg 4 hr pre	po	9.4-150	>150	-	>150	
1778	Mannozym	118	8-28-87	bid x 5, beg 4 hr pre	sc	1.6-100	>100	+	3.1	EXPANDED
1778	Mannozym	119	8-28-87	bid x 5, beg 4 hr pre	po	1.6-100	>100	-	>100	EXPANDED
1778	Mannozym	152	10-2-87	bid x 5, beg 4 hr pre	sc	6.25-100	>100	-	>100	BALLIET
1778	Mannozym	198	11-19-87	single, beg 24 hr pre	sc	6.3-50	>50	-	>50	
1778	Mannozym	199	11-19-87	single, beg 4 hr pre	sc	6.3-50	>50	-	>50	
1778	Mannozym	200	11-19-87	single, beg 4 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	201	11-19-87	single, beg 24 hr post	sc	6.3-50	>50	-	25	
1778	Mannozym	202	11-19-87	single, beg 48 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	203	11-19-87	single, beg 72 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	204	11-19-87	single, beg 96 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	216	12-4-87	qd x 5, beg 4 hr pre	sc	3.13-100	>100	±	3.13	
1778	Mannozym	217	12-4-87	bid x 5, beg 4 hr pre	ip	0.78-400	200	-	>100	
1778	Mannozym	239	01-08-88	qd x 5, beg 4 hr pre	sc	9.4-150	>150	?		
1778	Mannozym	250	01-15-88	single, beg 4 hr pre	sc	6.25-100	12.5	±	50	
1778	Mannozym	253	1-14-88	single	sc	6.75-100	>100	-	>100	IFN
1778	Mannozym	293	2-26-88	qd x 5, beg 4 hr pre	sc	9.4-150	>150	+	9.4	
1778	Mannozym	294	2-26-88	bid x 5, beg 4 hr pre	sc	1.6-50	>50	+	1.6	
1778	Mannozym	295	2-26-88	bid x 5, beg 24 hr post	sc	9.4-150	>150	+	1.6	
1778	Mannozym	296	2-26-88	bid x 5, beg 48 hr post	sc	9.4-150	>150	+	3.2	
1968	CL246,738	797	12-14-89	single, 4 hr pre	po	12.5-100	>100	+	12.5	EXPANDED
1968	CL246,738	798	12-14-89	3 shots, beg 24 hr post	po	12.5-100	>100	+	12.5	EXPANDED
1968	CL246,738	839	05-31-90	single, 4 hr pre	po	12.5-100	>100	-	>100	BALLIET
1969	CL259763	356	4-29-88	single, beg 24 hr pre	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	357	4-29-88	single, beg 4 hr pre	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	358	4-29-88	single, beg 24 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	359	4-29-88	single, beg 48 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	360	4-29-88	single, beg 72 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	391	6-9-88	single, beg 24 hr pre	ip	2, 20, 200	>200	±	20	
1969	CL259763	392	6-9-88	single, beg 4 hr pre	ip	2, 20, 200	>200	-	>200	
1969	CL259763	393	6-9 88	single, beg 48 hr post	ip	2, 20, 200	>200	-	>200	
1969	CL259763	394	6-9-88	single, beg 24 hr post	ip	2, 20, 200	>200	-	>200	
1969	CL259763	395	6-9-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	±	25	
1969	CL259763	425	7-1-88	bid x 5, beg 4 hr pre	po	2, 20, 200	>200	-	>200	
1969	CL259763	434	7-13-88	single, beg 24 hr pre	ip	5-80	>80	±	5	EXPANDED
1969	CL259763	436	7-13-88	eod x 3, beg 24 hr pre	ip	2-200	>200	-	>200	IFN
1969	CL259763	541	11-22-88	single, beg 4 hr post	ip	50-400	>400	-	>400	BALLIET
1976	Thymine riboside 2',3'-dialdehyde	446	7-21-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	-	>100	
1976	Thymine riboside 2',3'-dialdehyde	452	9-2-88	single, beg 24 hr pre	ip	62.5-500	500	±	62.5	
1976	Thymine riboside 2',3'-dialdehyde	453	9-2-88	single, beg 4 hr pre	ip	62.5-500	500	±	62.5	
1976	Thymine riboside 2',3'-dialdehyde	454	9-2-88	single, beg 24 hr post	ip	62.5-500	500	-	>500	
1976	Thymine riboside 2',3'-dialdehyde	481	9-30-88	bid x 5, beg 4 hr pre	ip	50-400	400	-	>400	
2149	Ampligen	56	3-12-87	qd x 8, beg 24 hr pre	ip	0.6-5	>5	+	0.625	
2149	Ampligen	57	3-12-87	eod x 8, beg 24 hr pre	sc	0.6-5	>5	+	0.625	
2149	Ampligen	69	3-26-87	qd x 8, beg 24 hr pre	sc	0.6-5	>5	+	0.313	EXPANDED
2149	Ampligen	128	9-10-87	qd x 5, beg 24 hr pre	ip	0.6-5	>5	+	0.625	
2149	Ampligen	129	9-10-87	qd x 5, beg 4 hr pre	ip	0.6-5	>5	+	0.625	

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2149	Ampligen	130	9-10-87	qd x 5, beg 4 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	131	9-10-87	qd x 5, beg 24 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	132	9-10-87	qd x 5, beg 48 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	142	9-25-87	qd x 5, beg 4 hr pre	po	0.04-5	>5	±	0.039	EXPANDED
2149	Ampligen	160	10-8-87	qd x 5, beg 4 hr pre	ip	0.625-5	>5	+	0.63	BALLIET
2149	Ampligen	166	10-16-87	qd x 5, beg 24 hr post	ip	0.05-5	>5	+	0.05	COMBINATION
2149	Ampligen	195	11-13-87	qd x 5, beg 24 hr post	ip	0.005	>0.005	±	0.005	COMBINATION
2149	Ampligen	205	11-20-87	bid x 5, beg 4 hr pre	ip	0.31-5	>5	+	0.625	
2149	Ampligen	207	12-4-87	qd x 5, beg 4 hr pre	ip	3.13-25	>25	+	3.13	TI
2149	Ampligen	208	12-4-87	single, beg 4 hr pre	ip	1.25-10	>10	+	1.25	
2149	Ampligen	209	12-3-87	single, beg 24 hr pre	ip	1.25-10	>10	+	1.25	
2149	Ampligen	210	12-4-87	single, beg 4 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	211	12-4-87	single, beg 24 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	212	12-4-87	single, beg 48 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	213	12-4-87	single, beg 72 hr post	ip	1.25-10	>10	-	>10	
2149	Ampligen	214	12-4-87	single, beg 96 hr post	ip	1.25-10	>10	-	>10	
2149	Ampligen	215	12-3-87	bid x 5, beg 24 hr pre	ip	0.6-5	>5	+	0.6	
2149	Ampligen	242	1-7-88	single	ip	0.05, 0.5, 5	>5	-		IFN
2149	Ampligen	257	01-22-88	qd x 5, beg 4 hr pre	ip	0.31-5	>5	+	0.31	
2149	Ampligen	309	3-11-88	qd x 5, beg 72 hr post	ip	0.625-5	>5	-	>5	
2149	Ampligen	310	3-11-88	qd x 5, beg 96 hr post	ip	0.625-5	>5	-	>5	
2149	Ampligen	362	5-6-88	bid x 5, beg 4 hr pre	ip	0.625-5	>5	-	>5	BALLIET
2149	Ampligen	407	6-17-88	qd x 5, beg 4 hr pre	ip	0.6-5	>5	-	>5	IFN
2149	Ampligen	408	6-17-88	single, beg 48 hr post	ip	0.6-5	>5	-	>5	IFN
2149	Ampligen	409	6-17-88	bid x 5, beg 4 hr pre	ip	0.6-5	>5	-	>5	IFN
2149	Ampligen	575	12-22-88	single, beg 4 hr pre	ip	0.63-5	>5	-	>5	BALLIET
2149	Ampligen	576	12-22-88	single, beg 4 hr post	ip	0.63-5	>5	±	0.63	BALLIET
2149	Ampligen	653	03-23-89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	654	03-23-89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	655	03-23-89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	656	03-23-89	qd x 5, beg 4 hr pre	ip	0.05-5	>5	+	0.05	MMF
2149	Ampligen	668	04-12-89	single, beg 48 hr post	ip	2.5	>2.5	ON TEST	ON TEST	IMMUNOLOGY
2149	Ampligen	673	04-27-89	3 in 7 days, beg 4 hr post	ip	0.125-1	>1	+	0.125	EXPANDED
2149	Ampligen	782	10-19-89	bid x 5, beg 4 hr pre	ip	0.6,5	>5	+	0.6	IFN
2149	Ampligen	783	10-19-89	eod x 3, beg 4 hr post	ip	0.6,5	>5	+	0.6	IFN
2149	Ampligen	784	10-19-89	single, beg 48 hr post	ip	0.6,5	>5	+	0.6	IFN
2149	Ampligen	786	10-19-89	qd x 5, beg 4 hr pre	ip	0.6,5	>5	+	0.6	IFN
2149	Ampligen	849	06-21-90	single, beg 23 hr post	ip	0.005-5	>5	+	0.005	COMBINATION
2276	Theracel No. BL-002	867	09-13-90	qd x 5, beg 4 hr post	po	10-500	>500	±	32	EXPANDED
2276	Theracel No. BL-002	879	10-17-90	qd x 5, beg 24 hr pre	po	125-2000	>2000	+	250	EXPANDED
2276	Theracel No. BL-002	881	10-22-90	qd x 1	po	125-2000	ON TEST	ON TEST	ON TEST	IFN
2285	Theracel No. BL-012	866	09-13-90	qd x 5, beg 4 hr post	po	10-500	>500	±	32	EXPANDED
2285	Theracel No. BL-012	880	10-17-90	qd x 5, beg 24 hr pre	po	125-2000	>2000	+	125	EXPANDED
2285	Theracel No. BL-012	882	10-22-90	qd x 1	po	125-2000	ON TEST	ON TEST	ON TEST	IFN
2700	6-Ethyl thiopurine riboside	432	7-14-88	bid x 5, beg 4 hr pre	ip	25-400	400	+	25	
2700	6-Ethyl thiopurine riboside	450	9-2-88	bid x 5, beg 4 hr pre	ip	3.13-100	>100	±	~50	EXPANDED
2700	6-Ethyl thiopurine riboside	473	9-22-88	bid x 5, beg 4 hr pre	ip	1.56-50	>50	±	~50	EXPANDED
2700	6-Ethyl thiopurine riboside	593	01-19-89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	12.5	EXPANDED

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2700	6-Ethyl thiopurine riboside	594	01-19-89	single, beg 4 hr pre	ip	31.3-500	>500	-	>500	
2700	6-Ethyl thiopurine riboside	595	01-19-89	single, beg 24 hr post	ip	31.3-500	>500	+	62.5	
2700	6-Ethyl thiopurine riboside	600	01-26-89	single, beg 4 hr pre	ip	31.3-500	250	-	>500	
2700	6-Ethyl thiopurine riboside	601	01-26-89	single, beg 24 hr post	ip	31.3-500	250	±	31.3	
2700	6-Ethyl thiopurine riboside	602	01-26-89	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	50	EXPANDED
2700	6-Ethyl thiopurine riboside	645	03-09-89	bid x 5, beg 24 hr pre	ip	9.4-150	>150	-	>150	BALLIET
2700	6-Ethyl thiopurine riboside	657	03-30-89	single, beg 24 hr post	po	31.3-500	>500	+	31.3	EXPANDED
2712	Bryostatin 1	305	3-4-88	qd x 5, beg 4 hr pre	ip	4.5-36	>36	±	18	
2712	Bryostatin 1	379	05-20-88	bid x 5, beg 4 hr pre	ip	6.25-50	>50	±	12.5	
2712	Bryostatin 1	426	7-1-88	qd x 5, beg 4 hr pre	ip	2.25-18 µg/ml	>18	-	>18	
2712	Bryostatin 1	503	10-20-88	qd x 5, beg 4 hr pre	ip	4.5-144 µg/ml	>144	-	>144	
2712	Bryostatin 1	509	10-26-88	single, beg 24 hr pre	ip	6.25-200 µg/ml	>200	+	12.5	
2712	Bryostatin 1	510	10-27-88	single, beg 4 hr post	ip	6.25-200 µg/ml	>200	+	12.5	
2712	Bryostatin 1	556	12-08-88	tid x 5, beg 4 hr pre	ip	1.13-18 µg/ml	>18	+	2.3	
2712	Bryostatin 1	565	12-15-88	single, beg 4 hr post	ip	6.25-100 µg/ml	>100	-	>100	EXPANDED
2713	Bryostatin 2	306	3-4-88	qd x 5, beg 4 hr pre	ip	4.5-36	>36	-	>36	
2713	Bryostatin 2	380	05-20-88	bid x 5, beg 4 hr pre	ip	5-40	>40	-	>40	
2716	UNIDENTIFIED	666	04-13-89	bid x 5, beg 4 hr pre	sc	18.8-300	>300	-	>300	
2741	Ribavirin tetrahydropyrimidine	149	10-2-87	bid x 5, beg 4 hr pre	sc	31.3-500	>500	-	>500	
2741	Ribavirin tetrahydropyrimidine	297	2-26-88	bid x 5, beg 4 hr pre	sc	75-600	>600	-	>600	
2742	Ribavirin 5-OH tetrahydropyrimidine	150	10-2-87	bid x 5, beg 4 hr pre	sc	31.3-500	>500	±	500	
2742	Ribavirin 5-OH tetrahydropyrimidine	607	02-09-89	single, beg 4 hr post	sc	31.3-500	>500	±	250	
2742	Ribavirin 5-OH tetrahydropyrimidine	608	02-09-89	single, beg 24 hr post	sc	31.3-500	>500	-	>500	
2776	Bropirimine	59	3-19-87	qd x 3, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	60	3-19-87	single, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	61	3-19-87	e 3 days x 3, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	90	4-23-87	single, beg 24 hr pre	ip	100-400	400	+	100	EXPANDED
2776	Bropirimine	143	9-25-87	single, beg 4 hr pre	ip	100-400	>400	+	100	
2776	Bropirimine	144	9-25-87	single, beg 4 hr post	ip	100-400	>400	+	200	
2776	Bropirimine	145	9-25-87	single, beg 24 hr post	ip	100-400	>400	+	200	
2776	Bropirimine	146	9-25-87	single, beg 48 hr post	ip	100-400	>400	+	400	
2776	Bropirimine	147	9-25-87	single, beg 72 hr post	ip	100-400	>400	-	>400	
2776	Bropirimine	148	9-25-87	single, beg 96 hr post	ip	100-400	>400	-	400	
2776	Bropirimine	254	1-21-88	qd x 3, beg 24 hr pre	po	25-400	400	+	25	EXPANDED
2776	Bropirimine	255	1-21-88	single, beg 24 hr pre	po	25-400	>400	+	25	EXPANDED
2776	Bropirimine	256	01-21-88	single, beg 24 hr pre	sc	50-400	200	+	200	
2776	Bropirimine	291	2-19-88	single, beg 24 hr post	po	25-100	>100	+	50	COMBINATION
2776	Bropirimine	312	3-11-88	qd x 3, beg 24 hr post	po	12.5-200	>200	+	12.5	EXPANDED
2776	Bropirimine	364	5-6-88	single, beg 4 hr pre	ip	50-400	>400	+	100	BALLIET
2776	Bropirimine	413	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	414	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	415	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	416	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	448	8-5-88	single, beg 24 hr post	po	400	>400	+	400	IFN
2776	Bropirimine	474	9-22-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	475	9-22-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	476	9-22-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	477	9-22-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
2776	Bropirimine	549	11-30-88	single, beg 48 hr post	ip	200	ON TEST	ON TEST	ON TEST	IMMUNOLOGY
2776	Bropirimine	573	12-22-88	single, beg 4 hr pre	ip	50-400	>400	+	50	BALLIET
2776	Bropirimine	574	12-22-88	single, beg4 hr post	ip	50-400	>400	-	>400	BALLIET
2776	Bropirimine	631	03-01-89	qd x 3, beg 24 hr pre	po	25-400	>400	+	25	EXPANDED
2776	Bropirimine	632	03-02-89	qd x 3, beg 4 hr post	po	25-400	>400	+	50	EXPANDED
2776	Bropirimine	633	03-01-89	qd x 3, beg 24 hr pre	ip	25-400	>400	+	25	
2776	Bropirimine	634	03-02-89	qd x 3, beg 4 hr post	ip	25-400	>400	+	25	
2776	Bropirimine	635	03-02-89	qd x 3, beg 24 hr post	ip	25-400	>400	+	50	
2776	Bropirimine	636	03-02-89	qd x 3, beg 24 hr pre	ip	62.5-1000	1000	+	62.5	BALLIET
2776	Bropirimine	637	03-08-89	single, beg 24 hr pre	po	25-800	>800	+	50	
2776	Bropirimine	638	03-09-89	single, beg 24 hr post	po	25-800	>800	+	100	
2776	Bropirimine	639	03-09-89	single, beg 48 hr post	po	25-800	>800	±	400	
2776	Bropirimine	640	03-09-89	single, beg 72 hr post	po	25-800	>800	-	>800	
2776	Bropirimine	641	03-08-89	eod x 3, beg 24 hr pre	po	25-400	>400	+	100	
2776	Bropirimine	642	03-08-89	e2d x 3, beg 24 hr pre	po	25-400	>400	+	50	
2776	Bropirimine	643	03-08-89	single, beg 24 hr pre	sc	25-400	>400	+	50	
2776	Bropirimine	644	03-08-89	bid x 3, beg 24 hr pre	ip	25-400	>400	+	200	
2776	Bropirimine	648	03-16-89	qd x 3, beg 24 hr post	po	25-100	>100	+	25	COMBINATION
2776	Bropirimine	658	03-29-89	single, beg 24 hr post	ip	200	ON TEST	ON TEST	ON TEST	IMMUNOLOGY
2776	Bropirimine	662	04-06-89	single, beg 4 hr pre	sc	25-400	>400	+	25	
2776	Bropirimine	663	04-05-89	eod x 3, beg 24 hr pre	po	50-400	>400	+	50	
2776	Bropirimine	664	04-05-89	etd x 3, beg 24 hr pre	po	50-400	>400	+	100	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	62	3-19-87	qd x 3, beg 24 hr pre	ip	50-400	400	+	200	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	63	3-19-87	single, beg 24 hr pre	ip	50-400	400	+	>400	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	64	3-19-87	e 3 days x 3, beg 24 hr pre	ip	50-400	>400	+	400	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	91	5-23-87	single, beg 24 hr pre	ip	100-400	200	±	100	EXPANDED
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	174	10-29-87	qd x 3, beg 24 hr pre	ip	37.5-300	>300	-	>300	BALLIET
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	231	12-10-87	qd x 3, beg 24 hr pre	po	50-400	200	+	50	EXPANDED
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	313	03-11-88	single, beg 4 hr pre	po	25-200	>200	±	25	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	65	3-26-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	66	3-26-87	single, beg 24 hr pre	ip	50-400	>400	+	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	67	3-26-87	e 3 days x 3, beg 24 hr pre	ip	50-400	>400	+	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	235	1-7-88	single, beg 24 hr pre	ip	50-800	>800	+	50	EXPANDED
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	274	2-4-88	single, beg 24 hr pre	po	50-400	200	+	50	EXPANDED
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	333	3-31-88	qd x 3, beg 24 hr pre	po	12.5-400	200	+	12.5	EXPANDED
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP	665	04-13-89	single, beg 24 hr post	po	12.5-200	>200	±	100	EXPANDED
2779	MVE-1	500	10-19-88	single, beg 24 hr pre	ip	6.25-100	>100	+	6.25	
2779	MVE-1	501	10-20-88	single, beg 4 hr pre	ip	6.25-100	>100	+	6.25	
2779	MVE-1	502	10-20-88	single, beg 24 hr post	ip	6.25-100	>100	+	12.5	
2779	MVE-1	543	11-22-88	single, beg 24 hr post	ip	12.5-100	>100	-	>100	BALLIET
2779	MVE-1	554	12-01-88	single, beg 4 hr pre	ip	0.78-100	>100	+	3.13	EXPANDED
2779	MVE-1	581	01-05-89	single, beg 24 hr post	ip	3.13-12.5	>12.5	±	6.25	COMBINATION
2779	MVE-1	585	01-13-89	qd x 3, beg 24 hr post	ip	1.56-50	>50	+	1.56	
2779	MVE-1	586	01-13-89	bid x 3, beg 24 hr post	ip	1.56-50	>50	-	>50	
2779	MVE-1	587	01-13-89	single, beg 24 hr post	sc	6.25-100	>100	+	6.25	
2779	MVE-1	588	01-13-89	single , beg 36 hr post	ip	6.25-100	>100	-	>100	
2779	MVE-1	589	01-13-89	single , beg 48 hr post	ip	6.25-100	>100	+	12.5	
2779	MVE-1	590	01-13-89	single, beg 4 hr pre	po	6.25-200	>200	-	>200	EXPANDED

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
2779	MVE-1	604	01-26-89	eod x 5, beg 4 hr pre	ip	3.13-100	>100	+	3.13	
2779	MVE-1	652	03-23-89	single, beg 4 hr pre	po	6.25-200	>200	±	25	EXPANDED
2779	MVE-1	660	04-06-89	single, beg 4 hr pre	po	9.4-150	>150	±	9.4	EXPANDED
2786	UNIDENTIFIED	667	04-13-89	bid x 5, beg 4 hr pre	sc	18.8-300	>300	-	>300	
2811	7-Deoxynarciclasine	236	01-08-88	qd x 5, beg 4 hr pre	ip	3.13-25	>25	-	>25	
2811	7-Deoxynarciclasine	369	05-13-88	bid x 5, beg 4 hr pre	ip	1-8	>8	±	4	
2812	Narciclasine	237	01-08-88	qd x 5, beg 4 hr pre	ip	0.75-6	>6	+	6	
2812	Narciclasine	292	2-26-88	qd x 5, beg 4 hr pre	ip	0.75-12	>12	+	0.75	EXPANDED
2812	Narciclasine	807	01-25-90	bid x 5, beg 4 hr pre	sc	0.75-25	6.25	±	3.13	EXPANDED
2812	Narciclasine	808	01-25-90	qd x 5, beg 4 hr pre	sc	0.75-25	6.25	+	0.75	EXPANDED
2812	Narciclasine	809	02-01-90	bid x 5, beg 4 hr pre	ip	0.195-3.13	3.13	-	>3.13	EXPANDED
2812	Narciclasine	810	02-01-90	qd x 5, beg 4 hr pre	ip	0.195-3.13	>3.13	+	0.78	EXPANDED
2880	Oxamisole	82	4-16-87	qd x 3, beg 24 hr pre	ip	1.6-25	>25	±	1.6	
2880	Oxamisole	83	4-16-87	qd x 3, beg 24 hr post	ip	1.6-25	>25	-	>25	
2880	Oxamisole	84	4-17-87	single, beg 24 hr post	ip	1.6-50	>50	±	25	
2880	Oxamisole	105	8-6-87	bid x 3, beg 24 hr pre	po	1.6-25	>25	±	1.56	EXPANDED
2880	Oxamisole	183	11-5-87	qd x 3, beg 24 hr pre	ip	1.55-25	>25	±	1.55	BALLIET
2880	Oxamisole	184	11-5-87	single, beg 24 hr pre	ip	3.13-50	50	±	25	BALLIET
2880	Oxamisole	206	11-19-87	bid x 3, beg 24 hr pre	ip	0.78-25	>25	±	1.56	
2880	Oxamisole	258	01-21-88	qd x 2, beg 24 hr pre	ip	0.78-50	50	±	0.78	
2880	Oxamisole	268	2-5-88	qd x 2, beg 4 hr pre	ip	0.78-50	>50	-	>50	
2880	Oxamisole	269	2-5-88	qd x 2, beg 4 hr post	ip	0.78-50	>50	±	25	
2880	Oxamisole	270	2-5-88	qd x 2, beg 24 hr post	ip	0.78-50	>50	-	>50	
2880	Oxamisole	271	2-5-88	qd x 2, beg 48 hr post	ip	0.78-50	>50	±	1.56	
2880	Oxamisole	272	2-4-88	e 3 day x 3, beg 24 hr pre	ip	0.78-50	>50	-	>50	
2880	Oxamisole	273	2-4-88	single	ip	3.13-50	>50	-	>50	IFN
2880	Oxamisole	334	4-1-88	qd x 3, beg 4 hr post	po	0.76-50	>50	±	0.76	
2880	Oxamisole	335	4-6-88	qd x 3, beg 24 hr pre	ip	1.5-25	>25	-	>25	
2885	3-T-butyl-1-adamantylthiourea	835	04-19-90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
2885	3-T-butyl-1-adamantylthiourea	841	06-07-90	bid x 5, beg 4 hr pre	sc	25-100	>100	±	25	EXPANDED
2885	3-T-butyl-1-adamantylthiourea	850	06-28-90	bid x 5, beg 4 hr pre	ip	75-600	>600	-	>600	
2933	CGP 19835 A Lipid	350	4-29-88	single, beg 48 hr pre	ip	10,100,1000µ	>1000 µg	±	1000	
2933	CGP 19835 A Lipid	351	4-29-88	single, beg 24 hr pre	ip	10,100,1000µ	>1000 µg	±	10	
2933	CGP 19835 A Lipid	352	4-29-88	single, beg 4 hr pre	ip	10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	353	4-29-88	single, beg 24 hr post	ip	10,100,1000µ	>1000 µg	+	100	
2933	CGP 19835 A Lipid	354	4-29-88	single, beg 48 hr post	ip	10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	355	4-29-88	single, beg 72 hr post	ip	10,100,1000µ	>1000 µg	±	1000	
2933	CGP 19835 A Lipid	402	6-9-88	eod x 3, beg 24 hr pre	ip	1,10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	410	6-17-88	single, beg 24 hr post	ip	313-10000µ	>10,000	+	313	EXPANDED
2933	CGP 19835 A Lipid	455	9-9-88	single, beg 24 hr post	sc	600-4800µ	>4800 µg	-	>4800	BALLIET
2933	CGP 19835 A Lipid	609	02-08-89	qd x 3, beg 24 hr pre	ip	1-1000µ	>1000 µg	+	100	
2933	CGP 19835 A Lipid	610	02-09-89	single, beg 24 hr post	po	1,10,100,1000µ	>1000 µg	-	>1000	EXPANDED
2933	CGP 19835 A Lipid	859	08-09-90	single, beg 4 hr post	ip	1250-10000 µ	>10000µg	-	>10000	BALLIET
2933	CGP 19835 A Lipid	860	08-09-90	single, beg 24 hr post	ip	1250-10000 µ	>10000µg	-	>10000	BALLIET
2978	Tetraacetate ester of 2980	298	2-26-88	bid x 5, beg 4 hr pre	sc	25-200	>200	±	50	
2978	Tetraacetate ester of 2980	332	4-1-88	bid x 5, 4 hr pre	ip	25-400	>400	-	>400	
2980	Tetrahydroxy analog of Pancratistatin	266	1-29-88	bid x 5, beg 4 hr pre	sc	31.3-500	31.3	-	>500	
2980	Tetrahydroxy analog of Pancratistatin	396	6-10-88	single, beg 4 hr pre	ip	6.25 50	>50	-	>50	

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
2980	Tetrahydroxy analog of Pancratistatin	397	6-10-88	single, beg 4 hr post	ip	6.25-50	>50	-	>50	
2980	Tetrahydroxy analog of Pancratistatin	398	6-10-88	single, beg 24 hr post	ip	6.25-50	>50	-	>50	
3425	8-Bromoguanosine	451	9-2-88	bid x 5, beg 4 hr pre	ip	15.6-500	500	-	>500	
3425	8-Bromoguanosine	491	10-12-88	single, beg 24 hr pre	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	492	10-12-88	single, beg 4 hr post	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	493	10-12-88	single, beg 24 hr post	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	505	10-27-88	qd x 5, beg 4 hr pre	sc	25-200	>200	±	50	
3425	8-Bromoguanosine	506	10-26-88	single, beg 24 hr pre	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	507	10-27-88	single, beg 4 hr post	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	508	10-27-88	single, beg 24 hr post	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	525	11-02-88	bid x 5, beg 24 hr pre	po	15.6-250	>250	-	>250	
3425	8-Bromoguanosine	526	11-09-88	single, beg 4 hr pre	sc	100-800	800	+	100	
3425	8-Bromoguanosine	527	11-10-88	single, beg 4 hr post	sc	100-800	800	+	100	
3425	8-Bromoguanosine	528	11-10-88	single, beg 24 hr post	sc	100-800	800	±	800	
3425	8-Bromoguanosine	564	12-08-88	qd x 5, beg 4 hr pre	sc	15.7-250	>250	-	>250	
3580	Sodium diethyldithiocarbamate	404	6-17-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	±	25	
3580	Sodium diethyldithiocarbamate	532	11-09-88	single, beg 24 hr pre	sc	37.5-300	>300	±	37.5	
3580	Sodium diethyldithiocarbamate	533	11-10-88	single, beg 4 hr post	sc	37.5-300	>300	-	>300	
3585	Neurotropin	126	9-3-87	twice 3 days sep., beg 24 pre	ip	3-24	>24	-	>400	
3585	Neurotropin	127	9-3-87	single, beg 24 hr pre	ip	3-24	>24	-	>400	
3585	Neurotropin	140	9-24-87	qd x 3, beg 24 hr pre	ip	3-24	>24	-	>24	
3585	Neurotropin	141	9-24-87	eod x 3, beg 24 hr pre	ip	3-24	>24	-	>24	
3585	Neurotropin	278	2-11-88	single, beg 24 hr pre	po	3-24		?		
3585	Neurotropin	316	03-17-88	single, beg 24 hr pre	po	3-24	>24	-	>24	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	120	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	400	-	>400	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	121	9-3-87	single, beg 24 hr pre	ip	50-400	400	+	100	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	399	6-10-88	single, beg 4 hr pre	ip	50-400	>400	-	>400	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	400	6-10-88	single, beg 4 hr post	ip	50-400	>400	+	100	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	401	6-10-88	single, beg 24 hr post	ip	50-400	>400	+	50	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	435	7-14-88	single, beg 4 hr post	ip	31.3-500	>500	±	31.3	EXPANDED
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	457	9-8-88	single, beg 4 hr post	po	31.3-500	500	±	125	EXPANDED
3588	Meta Fluoro ABPP	122	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	200	+	100	
3588	Meta Fluoro ABPP	123	9-3-87	single, beg 24 hr pre	ip	50-400	100	+	100	
3588	Meta Fluoro ABPP	175	10-29-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	50	BALLIET
3588	Meta Fluoro ABPP	281	2-12-88	single, beg 4 hr pre	ip	50-400	400	?		
3588	Meta Fluoro ABPP	282	2-12-88	single, beg 4 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	283	2-12-88	single, beg 24 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	284	2-12-88	single, beg 48 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	285	2-12-88	single, beg 72 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	286	2-12-88	single, beg 96 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	318	3-18-88	single, beg 4 hr pre	ip	50-400	400	±	<50	
3588	Meta Fluoro ABPP	319	3-18-88	single, beg 4 hr post	ip	50-400	400	+	100	
3588	Meta Fluoro ABPP	320	3-18-88	single, beg 24 hr post	ip	50-400	400	+	50	
3588	Meta Fluoro ABPP	321	3-18-88	single, beg 48 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	322	3-18-88	single, beg 72 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	323	3-18-88	single, beg 96 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	344	4-22-88	single, beg 4 hr pre	ip	37.5-300	300	±	75	
3588	Meta Fluoro ABPP	345	4-22-88	single, beg 4 hr post	ip	37.5-300	300	±	75	

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
3588	Meta Fluoro ABPP	346	4-22-88	single, beg 24 hr post	ip	37.5-300	300	+	37.5	
3588	Meta Fluoro ABPP	347	4-22-88	single, beg 48 hr post	ip	37.5-300	300	-	>300	
3588	Meta Fluoro ABPP	348	4-22-88	single, beg 72 hr post	ip	37.5-300	300	-	>300	
3589	5-Chloro-2,3-difluorophenyl ABPP	124	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	>400	+	200	
3589	5-Chloro-2,3-difluorophenyl ABPP	125	9-3-87	single, beg 24 hr pre	ip	50-400	400	-	>400	
3589	5-Chloro-2,3-difluorophenyl ABPP	176	10-29-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	400	BALLIET
3589	5-Chloro-2,3-difluorophenyl ABPP	458	9-7-88	qd x 3, beg 24 hr pre	po	31.3-500	>500	±	250	EXPANDED
3593	Ly 253,963	389	6-2-88	tid x 6, beg 24 hr pre	ip	1.2-150	>150	-	>150	
3593	Ly 253,963	390	6-2-88	bid x 6, beg 24 hr pre	ip	1.2-150	>150	-	>150	
3593	Ly 253,963	459	9-8-88	single, 24 hr pre	ip	31.3-500	>500	±	31.3	
3593	Ly 253,963	460	9-8-88	single, 4 hr post	ip	31.3-500	>500	±	31.3	
3593	Ly 253,963	461	9-8-88	single, 24 hr post	ip	31.3-500	>500	±	31.3	
3593	Ly 253,963	499	10-19-88	ad lib x 7, beg 4 hr pre drink	po	0.96-93	>93	±	0.96	EXPANDED
3679	1-(4-methoxybenzyloxy)adenosine perchloric acid salt	836	05-10-90	bid x 5, beg 4 hr pre	sc	25-400	>400	-	>400	INITIAL
3706	Tiazofurin triacetate	301	3-4-88	bid x 5, beg 4 hr pre	sc	56.3-450	>450	+	225	
3706	Tiazofurin triacetate	405	6-17-88	bid x 5, beg 4 hr pre	sc	75-600	>600	+	75	EXPANDED
3706	Tiazofurin triacetate	456	9-8-88	bid x 5, beg 24 hr pre	ip	100-800	>800	-	>800	BALLIET
3706	Tiazofurin triacetate	529	11-10-88	bid x 5, beg 4 hr pre	po	43.8-700	>700	+	175	EXPANDED
3925	du Pont A2222-1	189	11-12-87	single, beg 24 hr pre	ip	25-200	50	-	100	
3925	du Pont A2222-1	219	12-11-87	single, beg 4 hr pre	ip	25-200	50	-	>200	
3925	du Pont A2222-1	220	12-11-87	single, beg 4 hr post	ip	25-200	50	±	25	
3925	du Pont A2222-1	221	12-11-87	single, beg 24 hr post	ip	25-200	50	-	>200	
3925	du Pont A2222-1	222	12-11-87	single, beg 48 hr post	ip	25-200	50	-	>200	
3925	du Pont A2222-1	275	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	25	?		
3925	du Pont A2222-1	300	3-4-88	single, beg 4 hr pre	ip	3.13-25	>25	-	>25	
3925	du Pont A2222-1	406	6-15-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	±	6.25	
3925	du Pont A2222-1	441	7-20-88	3 times, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3925	du Pont A2222-1	442	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3925	du Pont A2222-1	530	11-09-88	single, beg 4 hr pre	ip	6.25-200	>200	+	6.25	EXPANDED
3925	du Pont A2222-1	531	11-10-89	single, beg 4 hr pre	ip	6.25-200	>200	-	>200	EXPANDED
3926	du Pont A2227-1	190	11-12-87	single, beg 24 hr pre	ip	25-200	25	-	25	
3926	du Pont A2227-1	223	12-11-87	single, beg 4 hr pre	ip	25-200	25	-	>200	
3926	du Pont A2227-1	224	12-11-87	single, beg 4 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	225	12-11-87	single, beg 24 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	226	12-11-87	single, beg 48 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	276	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3926	du Pont A2227-1	421	6-30-88	single, beg 24 hr pre	ip	12.5-100	>100	±	25	
3926	du Pont A2227-1	422	6-30-88	single, beg 4 hr pre	ip	12.5-100	>100	+	25	
3926	du Pont A2227-1	443	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3926	du Pont A2227-1	619	02-16-89	single, beg 4 hr pre	ip	3.2-50	>50	-	>50	EXPANDED
3927	du Pont A754-1	191	11-12-87	single, beg 24 hr pre	ip	25-200	100	-	100	
3927	du Pont A754-1	227	12-11-87	single, beg 4 hr pre	ip	25-200	200	-	>200	
3927	du Pont A754-1	228	12-11-87	single, beg 4 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	229	12-11-87	single, beg 24 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	230	12-11-87	single, beg 48 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	277	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3927	du Pont A754-1	315	3-16-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3927	du Pont A754-1	341	4-22-88	qd x 5, beg 24 hr pre	ip	3.13-25	>25	-	>25	

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AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
3927	du Pont A754-1	411	6-24-88	bid x 5, beg 24 hr pre	ip	3.13-25	>25	-	>25	
3927	du Pont A754-1	423	6-30-88	single, beg 24 hr pre	ip	25-200	>200	-	>200	
3927	du Pont A754-1	424	6-30-88	single, beg 4 hr pre	ip	25-200	>200	±	200	
3927	du Pont A754-1	444	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3933	Ge 089	303	3-3-88	qd x 5, beg 24 hr pre	ip	31.3-250	>250	-	>250	
3934	Ge 132, Germanium	192	11-12-87	qd x 7, beg 24 hr pre	po	9.4-300	>300	±	9.4	
3934	Ge 132, Germanium	218	12-10-87	qd x 7, beg 24 hr pre	ip	18.8-300	300	±	300	
3934	Ge 132, Germanium	367	5-6-88	bid x 7, beg 24 hr pre	ip	37.5-300	>300	+	37.5	
3934	Ge 132, Germanium	368	5-6-88	bid x 7, beg 4 hr pre	ip	37.5-300	>300	+	37.5	
3934	Ge 132, Germanium	387	6-3-88	bid x 5, beg 4 hr pre	ip	4.7-300	>300	±	4.7	EXPANDED
3934	Ge 132, Germanium	388	6-3-88	bid x 7, beg 4 hr pre	po	4.7-300	>300	±	18.8	EXPANDED
3934	Ge 132, Germanium	485	10-05-88	bid x 7, beg 24 hr pre	ip	18.8-600	>600	-	>600	EXPANDED
3934	Ge 132, Germanium	486	10-5-88	bid x 7, beg 24 hr pre	po	18.8-600	>600	-	>600	EXPANDED
3934	Ge 132, Germanium	487	10-5-88	bid x 7, beg 48 hr pre	po	18.8-600	>600	±	75	EXPANDED
3934	Ge 132, Germanium	515	10-26-88	single, beg 24 hr pre	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	516	10-27-88	single, beg 4 hr post	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	517	10-27-88	single, beg 24 hr post	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	542	11-22-88	single, beg 4 hr post	ip	100-800	>800	±	100	BALLIET
3934	Ge 132, Germanium	555	12-06-88	tid x 7, beg 48 hr pre	po	4.7-600	>600	±	37.5	EXPANDED
3934	Ge 132, Germanium	611	02-08-89	bid x 5, beg 24 hr pre	ip	37.5-600	>600	+	37.5	
3960	DMG	196	11-19-87	bid x 7, beg 36 hr pre	po	6.3-800	>800	-	>100	
3960	DMG	197	11-19-87	bid x 7, beg 36 hr pre	sc	6.3-800	>800	-	>100	
3960	DMG	279	2-11-88	bid x 7, beg 24 hr pre	ip	9.4-600	>600	?		
3960	DMG	349	4-22-88	bid x 5, beg 24 hr pre	sc	112.5-900	>900	-	>900	
4113	Pseudolycorine HCl	433	7-14-88	qd x 5, beg 4 hr pre	sc	0.75-12	>12	±	0.75	
4206	3-Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole	833	04-19-90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
4206	3-Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole	842	06-07-90	bid x 5, beg 4 hr pre	sc	25-100	>100	±	50	EXPANDED
4206	3-Acetamido-7-amino-6-methyl-7H-S-triazolo[5,1-C]-S-triazole	851	06-28-90	bid x 5, beg 4 hr pre	ip	75-600	>600	-	>600	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	862	09-06-90	bid x 5, beg 4 hr pre	sc	3.13 - 50	>50	-	>50	INITIAL
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	863	09-06-90	bid x 5, beg 4 hr pre	ip	3.13 - 50	25	+	3.13	INITIAL
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	868	09-20-90	single, beg 4 hr post	ip	12.5 - 200	25	±	12.5	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	870	09-20-90	single, beg 24 hr post	ip	12.5 - 200	25	-	>200	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	873	10-05-90	bid x 5, beg 4 hr pre	sc	03-13-50	50	+	3.13	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	874	10-05-90	bid x 5, beg 4 hr pre	ip	3.13 - 50	25	-	12.5	EXPANDED
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	875	10-05-90	bid x 5, beg 4 hr pre	po	3.13 - 50	>50	-	>50	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	888	11-01-90	single, 4 hr post	ip	1.56-12.5	>12.5	±	3.13	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	889	11-01-90	bid x 5, beg 4 hr pre	ip	0.8-6.25	>6.25	±	0.8	
4272	Trans-3-chloro-2-iodotetrahydrothiophene-1,1-dioxide	890	11-08-90	bid x 5, beg 4 hr pre	sc	06-25-50	>50	-	25	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	864	09-06-90	bid x 5, beg 4 hr pre	sc	6.25 - 100	>100	-	>100	INITIAL
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	865	09-06-90	bid x 5, beg 4 hr pre	ip	6.25 - 100	>100	+	6.25	INITIAL
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	869	09-20-90	single, beg 4 hr post	ip	12.5 - 200	>200	-	>200	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	871	09-20-90	single, beg 24 hr post	ip	12.5 - 200	>200	-	>200	
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	876	10-11-90	bid x 5, beg 4 hr pre	sc	6.25 - 100	100	-	>100	EXPANDED
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	877	10-11-90	bid x 5, beg 4 hr pre	ip	6.25 - 100	100	-	>100	EXPANDED
4273	2,3-Dihydro-5-iodothiphene-1,1-dioxide	878	10-11-90	bid x 5, beg 4 hr pre	po	6.25 - 100	>100	-	>100	
4282	AM-5	463	9-14-88	single, beg 24 hr pre	ip	12.5 200	12.5	-	<12.5	
4282	AM-5	464	9-14-88	single, beg 4 hr post	ip	3.125-50	3.125	-	3.125	
4282	AM-5	465	9-14-88	single, beg 24 hr post	ip	3.125 50	3.125	-	3.125	

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
4282	AM-5	494	10-12-88	single, beg 24 hr pre	ip	0.025-0.8	all lost wt	-	0.05	
4282	AM-5	495	10-12-88	single, beg 4 hr post	ip	0.025-0.8	all lost wt	±	0.025	
4282	AM-5	496	10-12-88	single, beg 24 hr post	ip	0.025-0.8	all lost wt	+	0.025	
4282	AM-5	552	12-01-88	single, beg 24 hr post	ip	0.025-0.8	0.4	+	0.025	EXPANDED
4282	AM-5	553	12-01-88	single, beg 48 hr post	ip	0.025-0.8	0.4	±	0.2	EXPANDED
4282	AM-5	571	12-14-88	eod x 3, beg 24 hr pre	ip	0.19-3	0.75	+	0.19	
4282	AM-5	572	12-14-88	qd x 3, beg 24 hr pre	ip	0.09-1.5	0.38	-	>1.5	
4282	AM-5	605	02-02-89	single, beg 4 hr pre	po	0.05-0.8	>0.8	+	0.05	EXPANDED
4282	AM-5	606	02-02-89	single, beg 24 hr post	po	0.05-0.8	>0.8	+	0.05	EXPANDED
4282	AM-5	616	02-17-89	single, beg 4 hr pre	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	617	02-17-89	single, beg 4 hr post	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	618	02-17-89	single, beg 24 hr post	ip	0.025-0.2	>0.2	-	>0.2	BALLIET
4282	AM-5	630	02-23-89	eod x 3, beg 24 hr pre	ip	0.025-0.8	0.8	+	0.025	EXPANDED
4282	AM-5	659	04-06-89	single, beg 24 hr post	ip	0.0031-0.05	>0.05	±	0.0031	
4283	AM-6	466	9-14-88	single, beg 24 hr pre	ip	12.5-200	all lost wt	+	12.5	
4283	AM-6	467	9-14-88	single, beg 4 hr post	ip	12.5-200	all lost wt	±	25	
4283	AM-6	468	9-14-88	single, beg 24 hr post	ip	12.5-200	all lost wt	+	12.5	
4284	AM-7	469	9-14-88	single, beg 24 hr pre	ip	11.25-80	>180	+	11.25	
4284	AM-7	470	9-14-88	single, beg 4 hr post	ip	11.25-80	>180	-	>180	
4284	AM-7	471	9-14-88	single, beg 24 hr post	ip	11.25-80	>180	±	22.5	
4285	AM-8	472	9-14-88	single, beg 24 hr pre	ip	6.25-100	>100	-	>100	
4285	AM-8	620	02-16-89	single, beg 24 hr post	ip	6.3-50	>50	Terminate	Terminate	TERMINATED
4285	AM-8	628	02-24-89	single, beg 24 hr post	ip	6.3-50	>50	+	25	
4286	P-136	488	10-5-88	single, beg 24 hr pre	ip	12.5-200	>200	+	12.5	
4286	P-136	489	10-5-88	single, beg 4 hr post	ip	12.5-200	>200	+	25	
4286	P-136	490	10-5-88	single, beg 24 hr post	ip	12.5-200	>200	+	12.5	
4287	P-117	478	9-21-88	single, beg 24 hr pre	ip	12.5-200	all lost wt	+	12.5	
4287	P-117	479	9-21-88	single, beg 4 hr post	ip	12.5-200	all lost wt	+	25	
4287	P-117	480	9-21-88	single, beg 24 hr post	ip	12.5-200	all lost wt	+	12.5	
4287	P-117	504	10-27-88	single, beg 24 hr post	ip	0.78-50	>50	+	0.78	EXPANDED
4588	1-aminoadenosinium mesitylenesulfonate	834	04-19-90	bid x 5, beg 4 hr pre	sc	25-400	>400	±	100	INITIAL
4588	1-aminoadenosinium mesitylenesulfonate	843	06-07-90	bid x 5, beg 4 hr pre	sc	25-100	>100	-	>100	EXPANDED
4588	1-aminoadenosinium mesitylenesulfonate	852	06-28-90	bid x 5, beg 4 hr pre	ip	75-600	>600	-	>600	
4593	P-188	482	9-29-88	single, beg 24 hr pre	ip	12.5-200	>200	+	12.5	INITIAL
4593	P-188	483	9-29-88	single, beg 4 hr post	ip	12.5-200	>200	±	12.5	INITIAL
4593	P-188	484	9-29-88	single, beg 24 hr post	ip	12.5-200	>200	±	12.5	INITIAL
4616	Noxymethyl pennicillinic acid	412	6-24-88	bid x 5, beg 4 hr pre	sc	18.8-150	>150	-	>150	INITIAL
4616	Noxymethyl pennicillinic acid	621	02-16-89	qd x 5, beg 4 hr pre	sc	25-200	>200	-	>200	
4616	Noxymethyl pennicillinic acid	622	02-16-89	single, beg 4 hr pre	sc	62.5-500	>500	Terminate	Terminate	TERMINATED
4616	Noxymethyl pennicillinic acid	623	02-16-89	single, beg 24 hr post	sc	62.5-500	>500	Terminate	Terminate	TERMINATED
4616	Noxymethyl pennicillinic acid	629	02-24-89	single, beg 24 hr post	sc	62.5-500	>500	-	>500	
4617	206-glycine	718	07-20-89	bid x 5, beg 4 hr pre	sc	50-800	>800	+	200	
4618	5'-N,N-diethylthiocarbamate-5'-deoxy-5'-thioadenosine	837	05-10-90	bid x 5, beg 4 hr pre	sc	25-400	.400	±	50	INITIAL
4618	5'-N,N-diethylthiocarbamate-5'-deoxy-5'-thioadenosine	853	06-28-90	bid x 5, beg 4 hr pre	sc	18439	>50	-	>50	EXPANDED
4726	CPG 19835 A Lipid - Placebo	462	9-8-88	single, beg 24 hr post	ip	undilute	no	-	>undilute	EXPANDED
5027	Imexon	699	07-07-89	qd x 5, beg 4 hr pre	ip	18.8-150	>150	-	>150	
5027	Imexon	700	07-07-89	qd x 5, beg 24 hr post	ip	18.8-150	>150	-	>150	
5054	1-[5'-(N-methyl-3-carbonyl-1,4-dihydropyridine)2',3'-bis-	612	02-15-89	single, beg 4 hr post	iv	4.3-34	34	-	>34	BALLIET

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
5055	UNIDENTIFIED	613	02-15-89	single, beg 4 hr post	iv	21.9-175	>175	-	>175	BALLIET
5056	UNIDENTIFIED	614	02-15-89	single, beg 4 hr post	iv	1.9-15	>15	-	>15	BALLIET
5057	UNIDENTIFIED	615	02-15-89	single, beg 4 hr post	iv	3.13-25	>25	-	>25	BALLIET
5079	Human Recombinant Interleukin II	758	09-14-89	qd x 5, beg 4 hr post	ip	1,563-25,000 cun	>25,000	+	1563	EXPND; IMMUN
5079	Human Recombinant Interleukin II	812	02-08-90	qd x 5, beg 4 hr post	ip	1,563-12,500 cun	>12,500	+	1563	EXPND; IMMUN
5221	Ribavirin 2'-3'-acetonide	582	01-11-89	single, beg 4 hr pre	iv	62.5-500	>500	-	>500	BALLIET
5222	2',3',N'-triisobutyrate-5',1,4-dihydrotri of AVS01	583	01-11-89	single, beg 4 hr pre	iv	1.95-15.6	>15.6	-	>15.6	BALLIET
5311	rIFN	789	11-09-89	single, beg 4 hr post	ip	10^3.5-10^5 upm	>10^5	+	4	EXPANDED
5311	rIFN	790	11-09-89	qd x 9, beg 4 hr post	ip	10^3.5-10^5 upm	>10^5	+	3.5	EXPANDED
5311	rIFN	826	04-05-90	qd x 5, beg 24 hr post	ip	10^3.5-10^5 upm	>10^5	+	5	EXPANDED
5311	rIFN	827	04-05-90	qd x 5, beg 36 hr post	ip	10^3.5-10^5 upm	>10^5	-	>10^5	EXPANDED
5311	rIFN	828	04-05-90	qd x 5, beg 48 hr post	ip	10^3.5-10^5 upm	>10^5	±	10^5	EXPANDED
5311	rIFN	840	05-31-90	qd x 8, beg 4 hr pre	ip	10^3.5-10^5 upm	>10^5	-	>10^5	BALLIET
5311	rIFN	858	07-26-90	qd x 5, beg 4 hr post	ip	10^3-10^5 upm	ON TEST	ON TEST	ON TEST	COMBINATION
5581	1-[5'-(1-methyl-3-carbonyl-1,4-dihydropyridine)2',3'-bis-O-	779	10-09-89	qd x 5, beg 4 hr post	iv,ip	31.3-125	>125	-	>125	BALLIET
5582	1-[5'-(1-methyl-3-carbonyl-1,4-dihydropyridine-ß-D-	780	10-09-89	qd x 5, beg 4 hr post	iv,ip	125-500	>500	-	>500	BALLIET
5587	7-Thia-8-oxoguanosine	674	05-03-89	2 times, 24 hr pre	ip	6.25-100	>100	+	50	EXPANDED
5587	7-Thia-8-oxoguanosine	675	05-04-89	2 times, 4 hr pre	ip	6.25-100	>100	+	6.25	EXPANDED
5587	7-Thia-8-oxoguanosine	676	05-04-89	2 times, 24 hr post	ip	6.25-100	>100	+	6.25	EXPANDED
5587	7-Thia-8-oxoguanosine	677	05-04-89	single, beg 24 hr post	ip	6.25-100	>100	+	25	EXPANDED
5587	7-Thia-8-oxoguanosine	757	09-08-89	2 shots, beg 36 hr post	ip	12.5-100	ON TEST	ON TEST	ON TEST	EXPANDED
5587	7-Thia-8-oxoguanosine	775	10-06-89	2 shots, 24, 31 hr post	ip	6.25-25	>25	+	12.5	COMBINATION
5587	7-Thia-8-oxoguanosine	872	09-20-90	2 shots, 24, 31 hr post	ip	25-50	>50	+	25	
5588	ICLC	679	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5588	ICLC	750	08-24-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5589	ICL-CMA	680	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5589	ICL-CMA	735	08-04-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	-	>0.1	EXPANDED
5590	ICL-CMD	681	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	0.25	EXPANDED
5590	ICL-CMD	743	08-10-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5591	ICL-CM-Beta-C-Dextrin	682	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5591	ICL-CM-Beta-C-Dextrin	744	08-10-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.0032	EXPANDED
5592	ICL-GEL	683	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5592	ICL-GEL	751	08-24-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.032	EXPANDED
5593	ICL-Sulfated Gel	684	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5593	ICL-Sulfated Gel	746	08-10-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.01	EXPANDED
5594	IC-(PLL-Dextran)	685	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5594	IC-(PLL-Dextran)	752	08-24-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	+	0.1	EXPANDED
5595	IC-(PLL-Dextran)	686	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	2.5	EXPANDED
5595	IC-(PLL-Dextran)	747	08-18-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	±	0.1	EXPANDED
5596	ICLC (heat cycled)	678	05-11-89	3 in 7 days, beg 4 hr post	ip	0.25, 1	>1	+	1	EXPANDED
5596	ICLC (heat cycled)	748	08-18-89	eod x 3, beg 4 hr post	ip	0.0032-0.1	>0.1	-	>0.1	EXPANDED
5786	UNIDENTIFIED	759	09-11-89	qd x 5, beg 4 hr post	iv,ip	4.0-16	>16	-	>16	BALLIET
5896	UNIDENTIFIED	760	09-11-89	qd x 5, beg 4 hr post	iv,ip	12.5-50	>50	±	50	BALLIET
5897	UNIDENTIFIED	781	10-16-89	qd x 5, beg 4 hr post	iv,ip	50-200	~100	-	>200	BALLIET
5898	UNIDENTIFIED	764	09-18-89	qd x 5, beg 4 hr post	iv,ip	12.5-50	>50	-	>50	BALLIET
6080	UNIDENTIFIED	795	12-11-89	qd x 5, beg 4 hr post	iv,ip	25-100	>100	±	50	BALLIET
6081	UNIDENTIFIED	796	12-11-89	qd x 5, beg 4 hr post	iv,ip	8 - 32	>32	-	>32	BALLIET
6082	UNIDENTIFIED	793	12-04-89	qd x 5, beg 4 hr post	iv,ip	18.8-75	>75	+	75	BALLIET

Table XIV-1. Punta Toro In Vivo Evaluations Dec. 1985-Nov. 1990

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox @	Results	MIC	Remarks
6083	UNIDENTIFIED	794	12-04-89	qd x 5, beg 4 hr post	iv,ip	8 0-32	>32	-	>32	BALLIET
6290	UNIDENTIFIED	805	01-22-90	qd x 5, beg 4 hr post	iv,ip	39 5-158	>158	-	>158	BALLIET
6291	UNIDENTIFIED	803	01-22-90	qd x 5, beg 4 hr post	iv,ip	12 5-50	>50	-	>50	BALLIET
6292	UNIDENTIFIED	804	01-22-90	qd x 5, beg 4 hr post	iv,ip	12 5-50	>50	-	>50	BALLIET
6297	UNIDENTIFIED	824	03-26-90	qd x 5, beg 4 hr post	iv,ip	6 25-25	>25	-	>25	BALLIET
6300	UNIDENTIFIED	825	03-26-90	qd x 5, beg 4 hr post	iv,ip	6 25 25	>25	-	>25	BALLIET
6334	UNIDENTIFIED	893	11-15-90	bid x 5, beg 4 hr pre	ip	7 8 250	>250	+	15 6	
6337	UNIDENTIFIED	894	11-15-90	bid x 5, beg 4 hr pre	ip	7 8 250	250	±	7 8	
6417	UNIDENTIFIED	895	11-15-90	bid x 5, beg 4 hr pre	ip	7 8-250	250	-	>250	
6477	UNIDENTIFIED	896	11-15-90	bid x 5, beg 4 hr pre	ip	3 2-100	>100	-	>100	
6501	UNIDENTIFIED	897	11-15-90	bid x 5, beg 4 hr pre	ip	7 8-250	>250	+	7 8	
01 + 2149	Ribavirin + Ampligen	163	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 5	+	0 32 + 5	COMBINATION
01 + 2149	Ribavirin + Ampligen	164	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 0 5	+	0 32 + 0 5	COMBINATION
01 + 2149	Ribavirin + Ampligen	165	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 0 05	+	0 32 + 0 05	COMBINATION
01 + 2149	Ribavirin + Ampligen	194	11-13-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150+0 005	+	0 32 + 0 005	COMBINATION
206 + 2776	Ribamidine + Bropirimine	288	2-19-88	206 bid x 5 2776 single, 24 post	po	2 4-75, 100	>75 + 100	+	2 4 + 100	COMBINATION
206 + 2776	Ribamidine + Bropirimine	289	2-19-88	206 bid x 5 2776 single, 24 post	po	2 4-75, 50	>75 + 50	+	2 4 + 50	COMBINATION
206 + 2776	Ribamidine + Bropirimine	290	2-19-88	206 bid x 5 2776 single, 24 post	po	2 4-75, 25	>75 + 25	+	2 4 + 25	COMBINATION
206 + 1767	Ribamidine + AM-3	383	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2 4-75, 50	>75 + 50	+	4 7 + 50	COMBINATION
206 + 1767	Ribamidine + AM-3	384	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2 4-75, 16	>75 + 16	+	4 7 + 16	COMBINATION
206 + 1767	Ribamidine + AM-3	385	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2 4-75, 5	>75 + 5	+	37 5 + 5	COMBINATION
01 + 1754	Ribavirin + MVE-2	428	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 5	>200 + 5	+	1 0 + 5	COMBINATION
01 + 1754	Ribavirin + MVE-2	429	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 0 5	>200 + 0 5	+	1 0 + 0 5	COMBINATION
01 + 1754	Ribavirin + MVE-2	430	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 0 05	>200 + 0 05	+	32 + 0 05	COMBINATION
01 + 2779	Ribavirin + MVE-1	578	01-05-89	01 bid x 5, 2779 single, 24 hr post	po,ip	1-300 + 12 5	>300+12 5	+	1 + 12 5	COMBINATION
01 + 2779	Ribavirin + MVE-1	579	01-05-89	01 bid x 5, 2779 single, 24 hr post	po,ip	1-300 + 6 25	>300+6 25	+	1 + 6 25	COMBINATION
01 + 2779	Ribavirin + MVE-1	580	01-05-89	01 bid x 5, 2779 single, 24 hr post	po,ip	1-300 + 3 13	>300+3 13	+	1 + 3 13	COMBINATION
01 + 2776	Ribavirin + Bropirimine	649	03-16-89	01 bid x 3, 2776 qd x 3, 24 hr post	po	3 13-1200+100	>1200+100	+	3 13 + 100	COMBINATION
01 + 2776	Ribavirin + Bropirimine	650	03-16-89	01 bid x 3, 2776 qd x 3, 24 hr post	po	3 13-1200+50	>1200+50	+	3 13 + 50	COMBINATION
01 + 2776	Ribavirin + Bropirimine	651	03-16-89	01 bid x 3, 2776 qd x 3, 24 hr post	po	3 13-1200+25	>1200+25	+	3 13 + 25	COMBINATION
01 + 5587	Ribavirin + 7-thia-8-oxoguanosine	776	10-06-89	01 bid x 3, 5587 2 shots, 24 hr post	po,ip	6 25-1250+25	1250+25	+	6 25+25	COMBINATION
01 + 5587	Ribavirin + 7-thia-8-oxoguanosine	777	10-06-89	01 bid x 3, 5587 2 shots, 24 hr post	po,ip	6 25-1250+12 5	1250+12 5	+	6 25+12 5	COMBINATION
01 + 5587	Ribavirin + 7-thia-8-oxoguanosine	778	10-06-89	01 bid x 3, 5587 2 shots, 24 hr post	po,ip	6 25-1250+6 25	1250+6 25	+	12 5+6 25	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	815	02-22-90	01 bid x 3, 1761 eod x 3, 24 hr post	po,ip	1 6-2000+0 32	2000+0 32	+	1 6+0 32	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	816	02-22-90	01 bid x 3, 1761 eod x 3, 24 hr post	po,ip	1 6-2000+0 01	2000+0 01	+	1 6+0 01	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	822	03-08-90	01 bid x 3, 1761 eod x 3, 24 hr post	po,ip	1 6-2000+0 0032	2000+0 0032	+	1 6+0 0032	COMBINATION
01 + 1761	Ribavirin + Poly ICLC	823	03-08-90	01 bid x 3, 1761 eod x 3, 24 hr post	po,ip	1 6-2000+0 001	2000+0 001	+	16+0 001	COMBINATION
01 + 2149	Ribavirin + Ampligen	845	06-21-90	01 bid x 3, 2149 single 23 hr post	po,ip	2.5-1500+5	1500+5	+	2 5+5	COMBINATION
01 + 2149	Ribavirin + Ampligen	846	06-21-90	01 bid x 3, 2149 single 23 hr post	po,ip	2 5-1500+0 5	1200+0 5	+	2 5+0 5	COMBINATION
01 + 2149	Ribavirin + Ampligen	847	06-21-90	01 bid x 3, 2149 single 23 hr post	po,ip	2 5-1500+0 05	1500+0 05	+	2 5+0 05	COMBINATION
01 + 2149	Ribavirin + Ampligen	848	06-21-90	01 bid x 3, 2149 single 23 hr post	po,ip	2 5-1500+0 005	>1500+0 005	+	2 5+0 005	COMBINATION
5587+antiIFN	7-Thia-8-oxoguanosine + anti-IFN	861	08-30-90	2 shots, 24 hr post, 24 5 hr post	ip	25-50 + 2000	>50+2000	+	25	COMBINATION
01 + 5311	Ribavirin + rHuIFN	856	07-26-90	01 bidx3 24 post, 5311 qdx5 4 post	po,ip	6 25-1500+10^4	ON TEST	ON TEST	ON TEST	COMBINATION
01 + 5311	Ribavirin + rHuIFN	857	07-26-90	01 bidx3 24 post, 5311 qdx5 4 post	po,ip	6 25-1500+10^3	ON TEST	ON TEST	ON TEST	COMBINATION

XV. PRESENTATIONS AND PUBLICATIONS

Presentations

1. Singh, V. K., R. W. Sidwell, and R. P. Warren. (1989) Immunologic properties of bropirimine in Punta Toro virus-infected mice. Abst. Intmtn. Br., Amer. Soc. Microbiol., p.1.
2. Barnard, D. L. and R. W. Sidwell. (1989) The effects of a *Phlebovirus* inhibitory agent, ribamidine, on cell proliferation and macromolecular synthesis in LLC-MK_2 cells. Abst. Intmtn. Br., Amer. Soc. Microbiol., p.1.
3. Sidwell, R. W. J. H. Huffman, H. Renis, M. Kende and J. Huggins. (1989) *In vivo* antiviral activity of bropirimine, an orally effective immunomodulator. Abstracts of the 89th Annual Meeting of the American Society for Microbiology, 1989, p. 29.
4. Barnard, D. L. and R. W. Sidwell. (1989) The effects of a *Phlebovirus* inhibitory agent, ribamidine, on cell proliferation and macromolecular synthesis in LLC-MK_2 cells. Abstracts of the 89th Annual Meeting of the American Society for Microbiology, 1989, p. 386.
5. Sidwell, R. W., J. H. Huffman, V. K. Singh, R. P. Warren, J. Coombs, R. Burger, M. Kende, and J. H. Huggins. (1989) Use of a murine Phlebovirus infection model for the evaluation of immunomodulating agents. Presented at a workshop of the International Conference on Comparative and Applied Virology, Banff, British Columbia, Canada, October, 1989.
6. Sidwell, R. W. (1989) A comparison of the anti-Punta Toro virus activity of ribavirin, ribavirin triacetate, and ribamidine. Seminar presented to USAMRIID, September 13, 1989.
7. Sidwell, R. W. (1989) In vivo antiviral experiences with combinations of immunomodulators and antiviral agents. Presented at the symposium, Immunomodulators as Antiviral Agents, International Congress of Chemotherapy, Jerusalem, Israel, June, 1989.
8. Sidwell, R. W., J. H. Huffman, V.K. Singh, R.P. Warren, J. Coombs, R. Burger, M. Kende, and J. Huggins. (1990) Use of the Punta Toro virus murine phlebovirus model for the evaluation of immunomodulating agents alone and in combination with antivirals. Presented at the UCLA Symposium Workshop, Animal Models of Human Viral Diseases: Relevance to Developmental Therapeutics. Keystone, Colorado.
9. Sidwell, R. W. (1990) Effect of drug combinations on in vivo Punta Toro virus infections in mice. Seminar presented to USAMRIID, May 22, 1990.
10. Sidwell, R. W., J. H. Huffman, V. K. Singh, R. P. Warren, R. Burger, L. Hall, J. Coombs, M. Kende, and J. H. Huggins. (1990) Antiviral activity of ampligen used alone and in combination with ribavirin. Am. Soc. Virology, Salt Lake City, UT, July 10, 1990.
11. Mead, J., R. Burger, Y. Yonk, J. Coombs, R. Warren, M. Kende, J. Huggins, and R. Sidwell. (1990) Effect of recombinant human interleukin-2 (rIL-2) on Punta Toro virus infections in C57BL/6 mice. Am. Soc. Virology, Salt Lake City, UT, July 10, 1990.
12. Coombs, J., R. W. Sidwell, J. Huffman, H. Renis, J. Huggins, and M. Kende. (1990) A comparison of pyrimidinone analog immunomodulators for treatment of Phlebovirus infections in mice. Intmtn. Amer. Soc. Microbiology, Pocatello, ID, April 21, 1990.
13. Smee, D. F., J. H. Huffman, J. Coombs, J. W. Huggins, and R. W. Sidwell. (1990) Effects of 7-thia-8-oxoguanosine alone and in combination with ribavirin on Punta Toro virus infections in mice. Third Internatl. Conf. on Antiviral Res. Abst. 153.
14. Sidwell, R. W. 1990. Synergistic use of ribavirin in combination with immune modulators for the treatment of *Phlebovirus* infections. Presented at the conference, "The Place of Ribavirin in the Therapy of HIV Infection" held at the Royal College of Physicians and Surgeons, Dublin, Ireland, Dec. 1, 1990.
15. Sidwell, R. W. 1991. Potential role of immunomodulators for treatment of phlebovirus infections of animals. Presented at the 1st Biennial Meeting of the American Society of Tropical Veterinary Medicine, San Juan, Puerto Rico, Feb. 8, 1991.

Publications

1. Sidwell, R. W., J. H. Huffman, B. B. Barnett, and D. Y. Pifat. 1988. *In vitro* and *in vivo phlebovirus* inhibition by ribavirin. Antimicrob. Ag. Chemother. 32:331-336.

2. Sidwell, R. W., J. H. Huffman, B. B. Barnett, M. Kende, and D. Y. Pifat. 1988. Effects of a series of immunomodulators on experimental *Phlebovirus* infections. Antiviral Res. 9:125.

3. Gabrielsen, B. J., M. A. Ussery, P. G. Canonico, G. R. Pettit, E. M. Schubert, and R. W. Sidwell. 1988. Anti-RNA-viral activities of phenanthridonis related to narciclasine. Antiviral Res. 9:97.

4. Sidwell, R. W., J. H. Huffman, D. L. Barnard, and D. Y. Pifat. 1988. Effects of ribamidine, a 3-carboxamidine derivative of ribavirin, on experimentally induced *phlebovirus* infections. Antiviral Res. 10:193-208.

5. Huffman, J. H., R. W. Sidwell, R. K. Robins, G. R. Revankar, and D. Y. Pifat. 1989. *In vitro* and *in vivo Phlebovirus* inhibition by nucleosides related to ribavirin. Nucleotides and Nucleosides 8:1159-1160.

6. Sidwell, R. W. 1989. *In vivo* antiviral experiences with combinations of immunomodulators and antiviral agents. *In*: Recent Adv. Chemotherapy (E. Rubinstein and D. Adam, eds.) pp. 713, 1-3. Lewin-Epstein Ltd., Jerusalem.

7. Smee, D.F., J.H. Huffman, L.L. Hall, J.W. Huggins, and R.W. Sidwell. 1990. Inhibition of *Phlebovirus* infections in vivo by tiazofurin and selenazofurin. Antiviral Research (in press).

8. Sidwell, R.W., J.H. Huffman, J. Coombs, H. Renis, J. Huggins, and M. Kende. 1990. A comparison of pyrimidinone analog immunomodulators for treatment of *Phlebovirus* infections in mice. Antiviral Chem. and Chemother. (in press).

9. Smee, D.F., J.H. Huffman, J. Coombs, J.W. Huggins, and R.W. Sidwell. 1990. Prophylactic and therapeutic activities of 7-thia-8-oxoguanosine against Punta Toro virus infections in mice. Antiviral Res. (in press).

10. Smee, D.F., H.A. Alaghamandan, J.H. Huffman, J. Coombs, J. Huggins, and R.W. Sidwell. 1990. Combination chemotherapy of alphavirus, bunyavirus, and flavivirus infections in mice using ribavirin and 7-thia-8-oxoguanosine. Antiviral Res. (in press).

11. Sidwell, R.W., J.H. Huffman, D.F. Smee, M. Kende, and J. Huggins. 1991. Synergistic use of ribavirin in combination with immune modulators for the treatment of *Phlebovirus* infections. In: Proc. Irish Conference on Ribavirin (Smith, R.E., ed.) Academic, NY (in press).

12. Bhagrath, M., R. Sidwell, K. Czako, K. Seyda, W. Anderson, N. Bodor, and M.E. Brewster. 1991. Improved delivery through biological membranes. 54. Synthesis, characterization an antiviral activity of a series of ribavirin chemical delivery systems: 5' and carboxamide derivatives. J. Med. Chem. (in press).

13. Deyrup, M., R. Sidwell, R. Little, P. Druzgala, N. Bodor, and M.E. Brewster. 1991. Improved delivery through biological membranes. 55. Synthesis and antiviral activity of a series of ribavirin chemical delivery systems: 2' and 3' derivatives. J. Med. Chem. (submitted).

14. Sidwell, R. W., J. H. Huffman, D. F. Smee, M. Kende, and J. Huggins. 1991. Synergistic use of ribavirin in combination with immune modulators for the treatment of *Phlebovirus* infections. *In:* The Place of Ribavirin in the Therapy of HIV Infection (R.A. Smith, ed.) Academic, New York City, (in press).